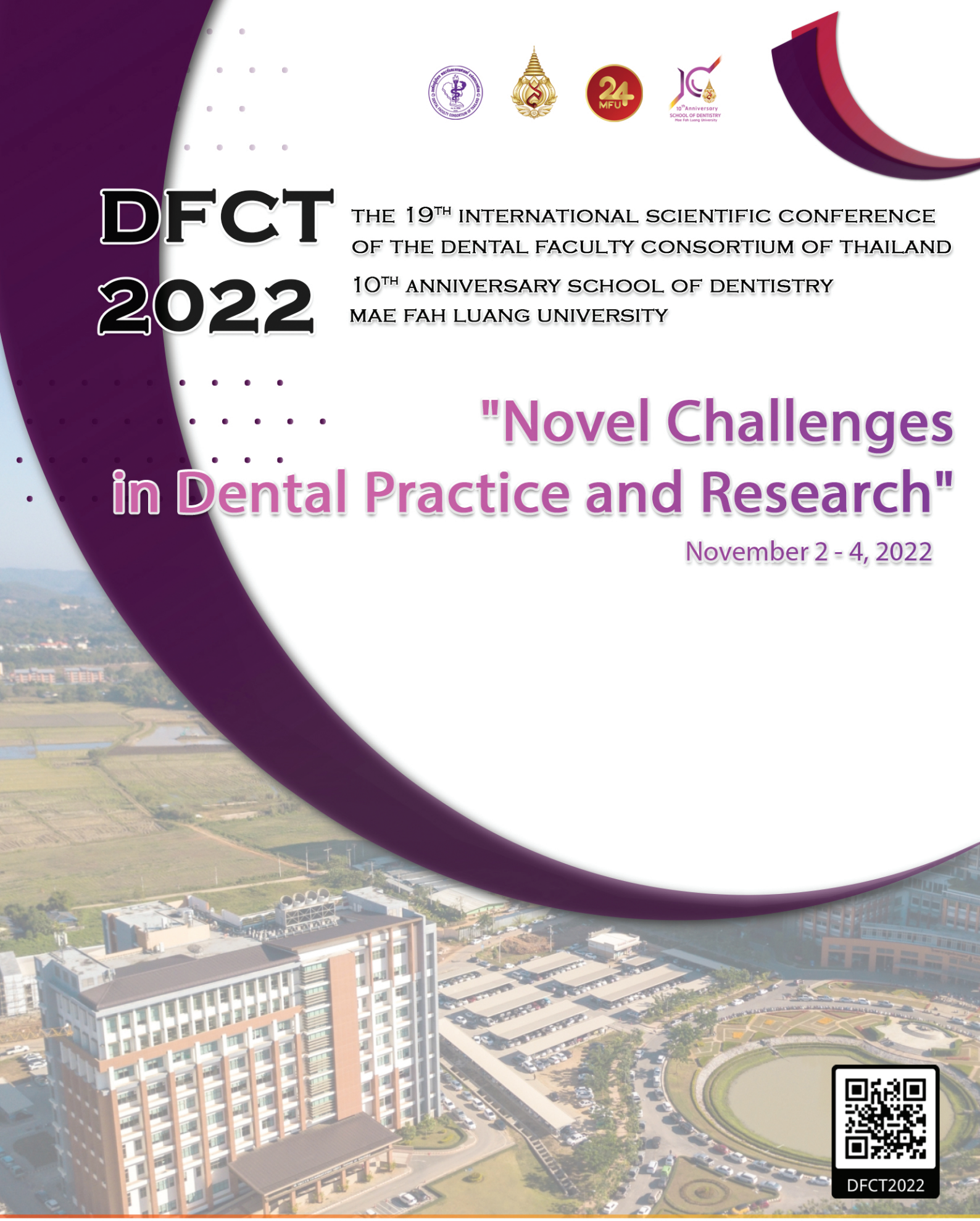
DFCT 2022

THE 19TH INTERNATIONAL SCIENTIFIC CONFERENCE
OF THE DENTAL FACULTY CONSORTIUM OF THAILAND
10TH ANNIVERSARY SCHOOL OF DENTISTRY
MAE FAH LUANG UNIVERSITY

"Novel Challenges in Dental Practice and Research"

November 2 - 4, 2022



DFCT2022

The 19th International Scientific Conference of the Dental Faculty Consortium of Thailand

DFCT2022

10th Anniversary School of Dentistry

Mae Fah Luang University

The 19th International Scientific Conference
of the Dental Faculty Consortium of Thailand (DFCT2022)
10th Anniversary School of Dentistry, Mae Fah Luang University

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"Novel Challenges in Dental Practice and Research"
November 2-4, 2022

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SCHOOL OF DENTISTRY
Mae Fah Luang University
DFCT2022
Chiang Rai, Thailand

Welcome Message

On behalf of president of Mae Fah Luang University, I would like to extend my congratulations to the School of Dentistry, Mae Fah Luang University for hosting the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand (DFCT 2022) on 2nd – 4th November 2022, and on the auspicious occasion to celebrate the 10th Anniversary of the School of Dentistry, Mae Fah Luang University. This year's event, entitled "Novel Challenge in Dental Practice and Research" aim to bring all dental faculties, researchers and clinicians in Thailand together to multiply and coordinate research and collaborate on the development of new technology as well as clinical care which will ultimately result in elevating the national's level of educational and oral health care.

I believe the conference will give participants a valuable platform to exchange and share new idea on various perspective of dental education and dental research among speakers and researchers. This is truly an opportunity to meet and interact with leading researchers friends and colleagues, as well as to discuss potential collaboration among participants.

Finally, I wish all participants a successful and achieve their goals and enjoy the beauty of Chiang Rai.

Sincerely yours,

Associate Professor Chayaporn Wattanasiri, Ph.D.
President
Mae Fah Luang University, Thailand

Welcome Message

หน่วยทันตกรรมพระราชทาน
ในพระบาทสมเด็จพระเจ้าอยู่หัว

It is my pleasure to welcome all esteemed guests and participants to the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand, DFCT 2022, held during November 2nd – 4th 2022, at School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand.

The Dental Faculty Consortium of Thailand (DFCT) was established in 1983. Currently DFCT consists of member from seventeen dental faculty and school in Thailand. The objective of DFCT is to continuously improve dental education and dental profession in Thailand. One of the activities we conduct is organizing scientific conference in order to exchange knowledge among dental professionals, dental students and other professionals.

As the Honorable Committee Member of the Dental Faculty Consortium of Thailand, I hope that this conference, titled “Novel Challenges in Dental Practice and Research” will provide you and update of new discoveries, enlightening discussion and valuable professional experiences from experts in various fields. Furthermore, participants may establish collaborative research network and be inspired to do translational researches to clinical practice and to create innovations.

I would like to extend my appreciation to outstanding international and Thai speaker for sharing their expertise with the participants, I also would like to take this occasion to congratulate School of Dentistry, Mae Fah Luang University on its 10th anniversary and acknowledge the school’s hosting this year conference.

I wish this conference the greatest success and excellence outcome.

Professor Thanpuying Petchara Techakampuch, D.D.S., M.S.
President of the Dental Innovation Foundation under the Royal Patronage (DIF)
Distinguished Committee Member of DFCT

Welcome Message

It is a great pleasure and honor for me to be invited to give a brief welcome message to guest speakers, participants, fellows and all students attending the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand (DFCT 2022). This year the Conference will be hosted by the Faculty of Dentistry, Mae Fah Luang University. Last year a similar conference entitled "Collaborative Research in Dentistry" and hosted by the Faculty of Dentistry, Chiang Mai University was highly successful. The theme for this year conference, ***Novel Challenges in Dental Practice and Research***, is equally attractive and interesting. It is particularly pleasing for me to see in the preliminary program for this year conference that a majority of the keynote lecture is geared toward a more basic aspect of dentistry, from molecular genetics of oral diseases and gene therapy to the effects of nutrition on oral health and ending up with dental education in the post COVID-19 era. All these lectures surprisingly coincide with my previous research interest on mucosal biology and immunity. I am looking forward to these talks as well as others to be presented in this conference. It is hoped that this type of conference will serve as forum for researchers, both local and abroad, to share and exchange their research experience and findings, and to establish future collaboration and networking. I am looking to witnessing the significant accomplishment by seniors and new generation researchers and wish all of them a highly successful research endeavor with bright future research career.

Last but not least, I would like to congratulate the School of Dentistry, Mae Fah Luang University on the auspicious occasion of its 10th anniversary.

Professor Stitaya Sirisinha, B.A., B.S., M.S., D.M.D., Ph.D.
Emeritus Professor of Microbiology
Faculty of Science, Mahidol University, Bangkok, Thailand
Distinguished Committee Member of DFCT

Welcome Message

It is my sincere pleasure to welcome you all to the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand held on 2nd – 4th November 2022, organized on this occasion to celebrate the 10th Anniversary of the School of Dentistry, Mae Fah Luang University. This conference theme, "Novel Challenge in Dental Practice and Research" bring together researcher in health science from the consortium member to meet, share and exchange their knowledge and expertise. In addition to many interesting will facilitate further development of dental research in Thailand, inspire young researcher and expand future collaboration.

I would like to thank the DFCT, School of Dentistry, Mae Fah Luang University

and organizing committee for organizing this conference.

Wish this conference very success and hope all participants have a successful outcome and enjoy the meeting.

Good luck to all.

Professor Viska Limwongse, D.D.S., M.S., Ph.D.
Distinguished Committee Member of DFCT

Welcome Message

It is my great honor and pleasure to welcome all speakers and participants to the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand at the School of Dentistry, Mae Fah Luang University during 2nd – 4th November 2022. This year theme "Novel Challenges in Dental Practice and Research" is very much interesting with the global challenges in many aspects.

I would like to take this opportunity to congratulate on the great success and continuing expansion of the School of Dentistry, Mae Fah Luang University on the auspicious occasion of the 10th anniversary.

I also believe that this conference will facilitate the dental academic member to develop more research and to inspire young researchers to enhance further collaboration.

I wish the conference great success and all participants having a wonderful time in Chiang Rai.

Associate Professor Surasith Kiatpongsan, D.D.S., M.S.
Distinguished Committee Member of DFCT

Welcome Message

It is a great pleasure and honor to welcome you all, invited speakers and participants, to the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand, DFCT 2022, held during November 2nd – 4th, 2022 at School of Dentistry, Mae Fah Luang University, Chaing Rai, Thailand.

I would also like to take this opportunity to express my sincere congratulation to our host, School of Dentistry, Mae Fah Luang University on the occasion of her 10th anniversary. I understand that this is the first international dental conference organized by School of Dentistry, Mae Fah Laung University, which I consider as an advance step to show her academic capacity at the international level even though just 10 years development.

The theme "Novel Challenge in Dental Practice and Research" is certainly relevant to the current global situation where innovation growing fast days after days, I certainly believe that the participants will have a good opportunity to exchange experience and learn from each other through many invited speakers and research presentations all around. Hopefully, the future dental practice will be enhanced for the benefit of your patients and people.

Recently, oral health has been well recognized at the World Health Organization and Global Oral Health Strategy was developed with Global Oral Health Action Plan is underway. More collaboration is therefore essential both nationally and internationally. Dental schools will definitely have certain roles in developing oral health workforces with appropriate technology for the benefit of their people in all age groups. This National Scientific Congress in Dentistry could be the initiation of collaboration between dental schools in the region sharing their expertise in dental education and technology with the aim of better oral health for all together.

For all participants, I recommend you to spend sometimes exploring the magnificent Chiangrai scenery and interesting artistic nice places.

May I wish the Conference a great success.

Associate Professor Prathip Phantumvanit, D.D.S., M.Sc., D.D.Sc.
Distinguished Committee Member of DFCT

Welcome Message

On behalf of the Dental Council of Thailand, I congratulate the School of Dentistry, Mae Fah Luang University on the 10th anniversary of the school's establishment. In the past 10 years, the school has succeeded in advancing her royal highness Princess Srinagarindra's royal wish to provide the people with good health and good teeth. The school enrolled its first class of dental students in 2012, and currently 4 classes have graduated and serve the dental needs of the people of Thailand, especially in the northern part of the country, with more graduates yet to come. This year the school also hosts the National Scientific Conference of the Dental Faculty Consortium of Thailand 2022, the theme of which is "Novel Challenges in Dental Practice and Research". There are many participants from around the country, as well as overseas academics attending this interesting event. At the same time they will enjoy the school tour to see its fully equipped clinics as well as the teaching program. I acknowledge the school for their efforts in hosting the scientific conference and wish for its fruitful outcome. Congratulations on the 10th anniversary of the School of Dentistry, Mae Fah Luang University and its successful mission.

Assistant Professor Suchit Poolthong, D.D.S., M.Sc., Ph.D.
President
The Dental Council of Thailand
Distinguished Committee Member of DFCT

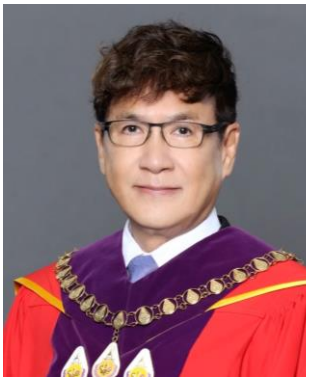
Welcome Message

As President of the Dental Association of Thailand, it's my pleasure to welcome you all to the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand (DFCT2022). The Dental Association of Thailand is proud to be a part of this important event. We have collaborations with the Deans in DFCT in bringing state-of-the-art knowledge to the dental community. This event held on 2-4 November 2022, marking the auspicious occasion of the 10th anniversary of the hosting institution – the School of Dentistry, Mae Fah Luang University. I would like to express my sincere congratulations to our host. This Dental School would continue to maintain its widely recognized reputation and provide excellence in dental education in the future.

The master theme "Novel Challenge in Dental Practice and Research" highlights the latest knowledge from biomedical sciences and dental public health to dental education impacted by the pandemic and other challenges. This Opportunity will bring together researcher in oral health science from consortium member. It will provide a platform for member of the consortium to meet, share and exchange their knowledge and expertise to improve the national and global oral health.

I would like to wish every participant a successful outcome of this conference and hope that you will all have a wonderful time and enjoy the natural beautiful environment of Chiang Rai.

Adirek Sriwatanawongsa, D.D.S.
President
The Dental Association of Thailand
Distinguished Committee Member of DFCT



Welcome Message

On behalf of the Royal College of Dental Surgeons of Thailand, I would like to congratulate The Dental Faculty Consortium of Thailand for the success in organizing their 19th International Scientific Conference during November 2-4, 2022 in Chiang Rai. I also would like to express my sincere gratitude and best wishes to School of Dentistry, Mae Fah Luang University for hosting this conference on the occasion of their 10th Anniversary.

This special event has included several renowned speakers both nationally and internationally to share their knowledge and experiences which would be very beneficial for all participants. The topics cover every aspects from basic research to clinical application under the theme "Novel Challenges in Dental Practice and Research." The Oral and Poster Presentation session give an opportunity for faculty members and students from every dental schools in Thailand to share their works and discuss their ideas with others.

As one of the main objectives for the Dental Faculty Consortium is to develop and improve dental education for the benefit of their students and the betterment of oral health for Thai population, I am delighted to find a whole day session prepared for Dental Education Seminar. I hope that the participants will be able to share their experiences from the recent pandemic situation and use this opportunity to change for better teaching and learning methods as well as improve students and teachers well-being. Every dental schools should be well prepared and ready for unexpected situation which may occur again in the future.

Finally, I wish this conference a great success with very fruitful result. I hope that all participants both local and from abroad will have a wonderful time both online and onsite. Please also stay safe everyone.

Emeritus Clinical Professor Passiri Nisalak, D.D.S., M.S.
President
The Royal College of Dental Surgeons of Thailand
Distinguished Committee Member of DFCT

Welcome Message

On behalf of Ministry of Public Health, it is my great pleasure to welcome all speakers and participants to the 19th International Scientific Conference of Dental Faculty consortium of Thailand (DFCT 2022) and congratulation to 10th Anniversary School of Dentistry, Mae Fah Luang University.

Under the theme of "Novel Challenges in Dental Practice and Research", the conference will provide a great opportunity for participant to update knowledge in several fields of interest such as microRNA-based gene therapy, host defense system, bone and dental pulp tissue engineering, fluoride vanish for geriatric dentistry and dental public health education. The field of new dental knowledge and innovation will serve a key foundation to develop new standards for Thailand's oral health service system.

Finally, I wish the conference great success and hope everyone enjoy the meeting.

Sura Wisedsak, M.D.
Deputy Permanent Secretary
The Ministry of Public Health
Distinguished Committee Member of DFCT

Welcome Message

It is a great pleasure and privilege to welcome all invited speakers and participants to the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand, DFCT 2022, with the theme "Novel Challenges in Dental Practice and Research"

This year's conference is truly special as it coincides with the auspicious 10th anniversary of our host the School of Dentistry, Mae Fah Luang University. Therefore, I would like to take this opportunity to express my sincere congratulations to our host and wish them continued success in the future.

I believe that the conference will provide more novel challenges and new concepts which will significantly benefit to the dental researchers and practitioners. I also would like to express my sincere thanks and appreciate to the Local Organizing Committee and everyone involved for the efforts and dedication in organizing this event. I wish this conference a great success.

Worawut Kulkaew, B.Eng., M.P.A.
Secretary General of the Dental Innovation Foundation under the Royal Patronage (DIF)
Distinguished Committee Member of DFCT

Welcome Message

Welcome to DFTC 2022, the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand. On behalf of the entire faculty of Dentistry here at Chiang Mai University, I congratulate Mae Fah Luang University on the 10th anniversary of the School of Dentistry. This is a milestone achievement marking the past and future commitments of the university and its school. Furthermore, I applaud Dean Paisan Kangvonkit and the entire School of Dentistry at Mae Fah Luang University for their leadership and hosting this auspicious event November 2nd-4th, 2022 in beautiful Chiang Rai, Thailand. I am confident all participants and attendees will discover the topic and shared experience of this hybrid event illuminating.

This year's topic, "Novel Challenges in Dental Practice and Research" offers insights in the new challenges facing dental practitioners, educators, and researchers. Topics such as microRNA-based gene therapy, promigratory molecules in oral cancer, and clinical applications in bone and dental pulp tissue engineering from presenters here and aboard extends a chance for presenters and attendees to learn, share and collaborate in an environment particularly suited for the advancement of dentistry and future research. The conference will present an important information exchange of research, experiences, developmental science, and technology in both a live and virtual setting.

Finally, let us all commemorate Mae Fah Luang University for their efforts in offering us all this opportunity to meet colleagues from across Thailand and the world. I would like to wish all guests, colleagues, speakers, presenters, and students attending the 19th annual conference at the School of Dentistry, Mae Fah Luang University, a prosperous future, and great success.

Enjoy the conference!

Assistant Professor Narumanas Korwanich, D.D.S, M.P.H., Ph.D.
President of the Dental Faculty Consortium of Thailand
Dean
Faculty of Dentistry, Chiang Mai University, Thailand

Welcome Message

On behalf of the Faculty of Dentistry, Chulalongkorn University, I would like to welcome all of you to the 19th Dental Faculty Consortium of Thailand's international dental academic conference and research presentation, on the subject of Novel Challenges in Dental Practice and Research, from the 2nd to the 4th of November, 2022, in conjunction with the School of Dentistry, Mae Fah Luang and the Dental Faculty Consortium of Thailand.

This is another opportunity to strengthen academic cooperation and tackle new challenges in the field of dentistry and research, with the goal of disseminating new knowledge and educating dental personnel on choosing the correct medical technology for treatment. We also hope that this conference will provide the opportunity for dental practitioners to share their knowledge and experiences, which can then be used to better treat their patients.

I would like to cordially invite professors, researches and academics to this academic conference, and sincerely hope the conference will be greatly beneficial to all who attends. I would also like to extend my special thanks to the professors, students and staffs that had helped make this conference a success.

I would like to express my sincere appreciation to the organizing committee and everyone involved with the 19th DFCT 2022 conference. Thank you very much. Please enjoy the conference.

Professor Pornchai Jansisyanont, D.D.S., M.Sc., Ph.D.
Dean
Faculty of Dentistry, Chulalongkorn University, Thailand

Welcome Message

It is my immense pleasure to cordially welcome you to the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand (DFCT 2022), held by the School of Dentistry, Mae Fah Luang University.

The conference this year is even more special as it coincides with the auspicious 10th anniversary of our esteemed host, the School of Dentistry, Mae Fah Luang University. Therefore, I would like to sincerely congratulate their faculty members, staff, and students on reaching this remarkable milestone, and wish them continued success in their future endeavors.

The selection of this year's theme "Novel Challenges in Dental Practice and Research", provides tremendous changes in many ways, especially to the dental research fraternity in which steps are taken beyond the normality to create a more holistic environment that ultimately translates efficiently for the benefit of the community. It is also a great opportunity for oral health professionals across Thailand and from other countries to share their unique experiences and insights into dental research.

I am confident that we can all contribute to creating a high-quality knowledge-sharing platform for the participating dentists, lecturers, researchers, and students, as well as expand our research collaboration to make remarkable advances by integrating applied and fundamental science for productive and efficient scientific research. Such collaboration is crucial for sustaining oral health and wellness within society.

I would also like to take this opportunity to extend my gratitude and appreciation to the local organizing committee, and everyone involved for the tireless effort and hard work in ensuring that this conference is conducted smoothly and successfully.

Your active participation is highly appreciated. I wish you all good health and an enjoyable event.

Sincerely,

Associate Professor Sirichai Kiattavorncharoen, D.D.S., M.D., Dr. Med.
Dean
Faculty of Dentistry, Mahidol University, Thailand

Welcome Message

On behalf of the Faculty of Dentistry, Khon Kaen University, it is my great pleasure to welcome all honorary speakers and participants to the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand (DFCT 2022), which will be held as a hybrid conference - virtually and on-site in Chiang Rai, during November 2 - 4, 2022. Importantly, I would like to extend my wholehearted congratulations to all staff, students and alumni of our prestigious host - the School of Dentistry, Mae Fah Luang University - for the celebration of the 10th anniversary of the school.

Under the theme of **"Novel Challenges in Dental Practice and Research"**, the conference will provide a great opportunity for lecturers, researchers, and students to update the enormous change in dental research and innovation. Moreover, this event represents a valuable platform for sharing new ideas and perspectives on various aspects of innovative dental practice, novel research as well as cutting-edge technologies among speakers and researchers from various countries and institutions. Furthermore, I truly believe that this remarkable event will also facilitate the dental academic members to develop more research and to inspire young researchers to enhance future collaborations.

Finally, I would like to express my sincere gratitude to the organizing committee and staff of the School of Dentistry, Mae Fah Luang University for their energetic efforts in organizing and making this conference to its milestone. I hope everyone has a successful outcome and enjoy the conference.

Associate Professor Waranuch Pitiphat, D.D.S., M.P.H.M., M.S., Sc.D., F.R.C.D.T.
Dean
Faculty of Dentistry, Khon Kaen University, Thailand

Welcome Message

On behalf of the Faculty of Dentistry. Prince of Songkla University. I would like to welcome you all to the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand (DFCT 2022) "Novel Challenges in Dental Practice and Research". More importantly, I congratulate the School of Dentistry, Mae Fah Luang University on its 10th anniversary and acknowledge the schools hosting this year conference.

Our collaborative efforts will enable us not only to achieve the goals of this meeting, that is the new challenges in dental practice and research, but also to give everyone the opportunity to exchange and learn from each other. Moreover, the involvement of creative ideas for innovation in all forms of research from valuable participants will definitely lead to the improvements in every aspect for our patients, as well as educational innovations for our dental students.

Thank you very much and enjoy the conference.

Professor Chidchanok Leethanakul, D.D.S., M.Sc., Ph.D.
Dean
Faculty of Dentistry, Prince of Songkla University, Thailand

Welcome Message

On behalf of the Faculty of Dentistry, Srinakharinwirot University, It is my distinct pleasure to warmly welcome you all to the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand (DFCT 2022), in the topic "Novel Challenges in Dental Practice and Research" which will be held at School of Dentistry, Mae Fah Luang University, in Chiang Rai province, on November 2-4, 2022. Moreover, it is my great honor to congratulate in advance for the 10th anniversary of School of Dentistry, Mae Fah Luang University establishment.

This special annual event is also recognized for bringing the participants to exchange ideas, knowledge and discover novel opportunities and broaden their knowledge for the current state of dental education that useful for the delegates in academia. Finally, I would like to give my special thanks to the organizing committees, School of Dentistry, Mae Fah Luang University for organizing this conference.

I wish this conference the greatest success and excellence outcome that all participants will enjoy the quality schedule and beautiful conference's venue.

Assistant Professor Nathawut Kaewsutha, D.D.S., M.P.H., Ph.D.
Dean
Faculty of Dentistry, Srinakharinwirot University, Thailand

Welcome Message

On behalf of the Faculty of Dentistry, Thammmasat University, I would like to express my sincere gratitude and congratulate the School of Dentistry Mae Fah Luang University, for hosting the 19th International Scientific Conferences of The Dental Faculty Consortium of Thailand during November 2-4, 2022. As the part of DFCT which is a leading organization that has been extensively involved in the development of dental education. This conference under the theme "Novel Challenges in Dental Practice and Research" will provide a great opportunity for the experts to share their immense knowledge, skill and experience among the various members of the dental profession. It is also dedicated to provide a great opportunity for the networking and exchange of ideals with participants from all dental schools in Thailand and oversea with the aim of better clinical practice and oral health for all together.

I would like to take this opportunity to salute the Dean, faculty and supporting staffs from Mae Fah Luang University, for their hard works and efforts to organize this vibrant conference also an experienced speakers and presenters who making this conference a successful and remarkable event

All the best for your success.

Associate Professor Samroeng In-gram, D.D.S., Ph.D.
Dean
Faculty of Dentistry, Thammasat University, Thailand

Welcome Message

On behalf of Faculty of Dentistry, Naresuan University, we are honored and warmly welcome you to the 19th international scientific conference of the Dental Faculty Consortium of Thailand (DFCT2022). We are certain that this will be a platform to gather and disseminate the latest knowledge in recent advancements in emerging areas of diversified research fields covered during a conference that will provide a platform for the participants to share experiences and knowledge through several sessions.

The success of the conference depends on many people who have worked in planning and organizing. Many thanks go out to the members of the DFCT, the Organizing Committee and the Steering Committee who have all worked extremely hard for their input and support. In particular, we would like to thank all of the conference participants for their contributions which are the foundation of this conference. We hope you will be appreciated the little things of the event.

Associated Professor Anuphan Sittichokechaiwut, D.D.S., Ph. D.
Dean
Faculty of Dentistry, Naresuan University, Thailand

Welcome Message

I am delighted to welcome all the distinguished speakers, dentists, researchers, and dental students from all parts of the world to the 19th DFCT International Scientific Conference of the Dental Faculty Consortium of Thailand, hosted by School of Dentistry, Mae Fah Luang University. This conference is a great platform to broaden new knowledge, exchange valuable experiences, as well as establish contacts between participants. I certainly believe that this 19th DFCT International Scientific Conference will be fruitful results and lay the groundwork for the development in the field of dentistry.

Clinical Professor Tasanee Tengrungsun, D.D.S.
Dean
School of Dentistry, University of Phayao, Thailand

Welcome Message

On behalf of the Institute of Dentistry, Suranaree University of technology (SUT), this is my great honors and pleasure to welcome all speakers and participants to the 19th DFCT 2022 and celebrating 10th Anniversary School of Dentistry, Mae Fah Luang University at Chiang Rai province, Thailand.

Under the theme of "Novel Challenges in Dental Practice and Research", participants will have the opportunity to update, discuss, and share experiences with expert researchers from national and international institutes on new challenges of dental practices and cutting-edge research.

I would like to express my appreciation to the faculties and staffs at School of Dentistry, Mae Fah Luang University for hosting this conference, organizing committee and making this successful event.

Finally, I wish all attendants enjoy this successful and fruitful conference that will benefit to your research and career.

Sincerely,

Professor Pasutha Thunyakitpisal, D.D.S., Ph.D.
Acting Dean
Institute of Dentistry, Suranaree University of Technology, Thailand

Welcome Message

I would like to personally congratulate the success of this conference, The Dental Faculty Consortium of Thailand and the 10th anniversary of the School of Dentistry, Mae Fah Luang University The meeting is the result of the success as is evident from the invited keynote speakers, lecturers, and experts from Thailand and various countries.

This meeting is also the result of the diligence and dedication of our postgraduates and staff and their papers. This wonderful opportunity allows us to come together to discuss the developments in dental practice and research and this meeting is an excellent platform where we can share practical knowledge openly which will help us to continue improving our dental research.

Sincerely,

Professor Sittichai Koontongkaew, D.D.S., Ph.D.
Dean
International College of Dentistry, Walailak University, Thailand

Welcome Message

On behalf of the college of Dental Medicine, Rangsit University. I am delighted to warmly welcome you all to the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand, DFCT 2022, in the theme "Novel Challenges in Dental Practice and Research" during November 2nd – 4th, 2022. This is the annual scientific conference of all dental schools in Thailand to update and share their research interest in all area of dentistry and health science.

I would like to take this opportunity to express my sincere congratulation to our host, School of Dentistry, Mae Fah Laung University, on the occasion of it's 10th anniversary. I am confident that the institute would continue to maintain its widely recognition and provide excellence in dental education and research.

I also believe that all the participants will have a great opportunity to exchange research and academic knowledge and gain new technology in oral health

Finally, I wish the conference great success and hope you will enjoy and having a pleasant stay in Chiang Rai.

RADM Suchada Vuddhakanok, R.T.N., D.D.S., M.S.
Dean
College of Dental Medicine, Rangsit University, Thailand

มหาวิทยาลัยเวสเทิร์น
WESTERN UNIVERSITY

Welcome Message

On behalf of the Faculty of Dentistry, Western University, I would like to extend my warmest congratulations to the 19th International Scientific Conference of Dental Faculty Consortium of Thailand (DFCT 2022) which will be held on November 2nd – 4th, 2022, organized by the School of Dentistry, Mae Fah Luang University.

As the transition to hybrid scientific events continues, we undeniably face the new challenge of balancing the utilization of rapidly changing technologies with safe and sustainable dental practices. Consequently, this year's theme of "Novel Challenges in Dental Practice and Research" represents an ideal opportunity for each and every researcher to bring their innovative approaches, expertise, and insights from latest research findings in their respective fields to share with their colleagues in this leading conference so that we can collectively identify and find new solutions to these challenges.

Once again, I would like to take this opportunity to express my sincere thanks to all contributing experts, researchers, and other participants for their time, expertise, and valuable insights. I wish the DFCT 2022 conference great success and extraordinary results.

Sincerely

Professor Vinai Sirichitra, D.D.S., M.S.
Dean
Faculty of Dentistry, Western University, Thailand

Welcome Message

I would like to extend my congratulation to the School of Dentistry, Mae Fah Luang University, for hosting the 19th International Scientific Conference of the Faculty Consortium of Thailand and welcome all attendees to the city of Chiang Rai.

The theme of this conference, "Novel Challenges in Dental Practice and Research" will be beneficial to the progress of dental technology and science. Integrative knowledge has become the new paradigm, and thus, the collaboration between research groups is critical.

On behalf of the dean of the Faculty of Dentistry, Bangkokthonburi University, I also would like to extend my appreciation for the invaluable opportunities given to our graduate students to participate in this conference as attendees and presenters. I wish for a successful conference and look forward to seeing everyone.

Sincerely,

Associate Professor Thongnard Kumchai, D.D.S.
Dean
Faculty of Dentistry, Bangkokthonburi University, Thailand

Nation University
มหาวิทยาลัยเนชั่น

Welcome Message

"Novel Challenges in Dental Practice and Research", an annual symposium that is valuable both for the dental profession and those interested in other fields, has contributed to the development of knowledge and ideas for developing ways to promote a better quality of life for people.

On behalf of the Faculty of Dentistry, Nation University Congratulations and thank you for letting us be a part of this great conference, the 19th International Scientific Conference of the Dental Faculty of Thailand (DFCT 2022).

Congratulations to the participants of this great academic conference have brought this invaluable knowledge and experience used to extend knowledge and abilities to be more successful.

Sincerely yours,

Siriporn Sasakul, D.D.S.
Acting Dean
Faculty of Dentistry, Nation University, Thailand

Welcome Message

On behalf of the School of Dentistry, King Mongkut's Institute of Technology Ladkrabang, I would like to welcome all of you to the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand (DFCT 2022), held by School of Dentistry, Mae Fah Luang University on November 2-4, 2022. This year is especially auspicious as it marks the 10th year anniversary of the School of Dentistry, Mae Fah Luang University

The Scientific Conference's theme "Novel Challenges in Dental Practice and Research" provides various advanced knowledge, research and cutting-edge dental technologies by international and national speakers. This conference will certainly be a venue for dental researchers and academics as well as dental students to get together to share their ideas for future collaboration.

I would like to sincerely congratulate to the local organizing committee, and staffs for the hard work in successfully managing this conference.

Sincerely,

Associate Professor Araya Phonghanyudh, D.D.S., Ph.D.
Dean
School of Dentistry, King Mongkut's Institute of Technology Ladkrabang, Thailand

Welcome Message

It is a great honor for the School of Dentistry, Mae Fah Luang University, to be hosting the 19th International Scientific Conference of the Dental Faculty Consortium of Thailand (DFCT 2022) on November 2-4, 2022. This year is especially auspicious as it marks the 10th year anniversary of the School of Dentistry. This year's topic is "Novel Challenges in Dental Practice and Research". The conference will be held both online and onsite.

On this occasion and on behalf of the School of Dentistry, Mae Fah Luang University, I would like to welcome all the members, delegates, speakers, and participants from 17 Thai dental schools to this scientific conference. My hearty welcome is also extended to participants from dental schools abroad.

The conference is full of academic activities because several world-renowned keynote speakers, both local and international, from various disciplines, such as bone and pulp tissue engineering, periodontal innate host defense, microRNA-based gene therapy, and regenerative endodontics, will present you with frontier research as well as cutting-edge technologies.

I envision that this scientific conference will be a venue for enriching discussion and for dental researchers to get together to share their ideas for future collaborative research.

I also hope and recommend that you take advantage of the nice weather in Chiang Rai to sightsee and experience the northern Thai culture and warm hospitality. I look forward to greeting you all in Chiang Rai.

Assistant Professor Paisan Kangvonkit, D.D.S., M.S.
Dean
School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand

Organizing Committee

คำสั่งมหาวิทยาลัยแม่ฟ้าหลวง

ที่ ๗๘๐๔ / ๒๕๖๕

เรื่อง แต่งตั้งคณะอนุกรรมการจัดการประชุมวิชาการและเสนอผลงานวิจัยสาขาทันตแพทยศาสตร์ ครั้งที่ ๑๙ องค์กรผู้บริหารคณะทันตแพทยศาสตร์แห่งประเทศไทย และการประชุมวิชาการฉลองครบรอบ ๑๐ ปี สำนักวิชาทันตแพทยศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง ระหว่างวันที่ ๒ – ๔ พฤศจิกายน ๒๕๖๕ ณ มหาวิทยาลัยแม่ฟ้าหลวง

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ด้วยองค์กรผู้บริหารคณะทันตแพทยศาสตร์แห่งประเทศไทย ซึ่งประกอบด้วยผู้ทรงคุณวุฒิ ทันตแพทยสมาคมแห่งประเทศไทย ทันตแพทยสภา ราชวิทยาลัยทันตแพทย์แห่งประเทศไทย และสถาบันทันตแพทยศาสตร์ ทั้ง ๑๖ สถาบัน ได้แก่ มหาวิทยาลัยขอนแก่น จุฬาลงกรณ์มหาวิทยาลัย มหาวิทยาลัยเชียงใหม่ มหาวิทยาลัยเทคโนโลยีสุรนารี มหาวิทยาลัยธรรมศาสตร์ มหาวิทยาลัยนเรศวร มหาวิทยาลัยพะเยา มหาวิทยาลัยมหิดล มหาวิทยาลัยแม่ฟ้าหลวง มหาวิทยาลัยศรีนครินทรวิโรฒ มหาวิทยาลัยสงขลานครินทร์ มหาวิทยาลัยวลัยลักษณ์ สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกระบัง มหาวิทยาลัยรังสิต มหาวิทยาลัยเวสเทิร์น มหาวิทยาลัยกรุงเทพธนบุรี และมหาวิทยาลัยเนชั่น ได้มีมติให้สำนักวิชาทันตแพทยศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง เป็นเจ้าภาพจัดประชุมวิชาการและเสนอผลงานวิจัย สาขาทันตแพทยศาสตร์ ครั้งที่ ๑๙ องค์กรผู้บริหารคณะทันตแพทยศาสตร์แห่งประเทศไทย ในระหว่างวันที่ ๒ – ๔ พฤศจิกายน ๒๕๖๕

เพื่อให้การดำเนินงานดังกล่าวเป็นไปด้วยความเรียบร้อย อาศัยอำนาจตามความมาตรา ๒๖ แห่งพระราชบัญญัติมหาวิทยาลัยแม่ฟ้าหลวง พ.ศ. ๒๕๔๑ จึงเห็นควรแต่งตั้งคณะอนุกรรมการจัดการประชุมวิชาการและเสนอผลงานวิจัยสาขาทันตแพทยศาสตร์ ครั้งที่ ๑๙ องค์กรผู้บริหารคณะทันตแพทยศาสตร์แห่งประเทศไทย และการประชุมวิชาการฉลองครบรอบ ๑๐ ปี สำนักวิชาทันตแพทยศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง ระหว่างวันที่ ๒ – ๔ พฤศจิกายน ๒๕๖๕ ณ มหาวิทยาลัยแม่ฟ้าหลวง ดังต่อไปนี้

องค์ประกอบ

๑. คณะอนุกรรมการฝ่ายจัดงาน พิธีการ และประสานงานทั่วไป

๑. รองอธิการบดี (รองศาสตราจารย์ ดร.นันทนา คชเสนี) ที่ปรึกษา

๒. รองอธิการบดี (ศาสตราจารย์ ดร.สุจิตรา วงศ์เกษมจิตต์) ที่ปรึกษา

/๓. รองอธิการบดี...

๓. รองอธิการบดี (อาจารย์ ดร.ต่อพันธ์ ทันดร)	ที่ปรึกษา
๔. รองอธิการบดี (อาจารย์ ดร.พรรณรวี พรหมนารท)	ที่ปรึกษา
๕. คณบดีสำนักวิชาทันตแพทยศาสตร์	ประธานอนุกรรมการ
๖. อาจารย์ ทันตแพทย์พิศักดิ์ องค์ศิริมงคล	รองประธานอนุกรรมการ
๗. ผู้อำนวยการศูนย์บริการวิชาการ	อนุกรรมการ
๘. ผู้อำนวยการศูนย์บริการเทคโนโลยีสารสนเทศ	อนุกรรมการ
๙. ผู้อำนวยการสถาบันวิจัยและนวัตกรรมมหาวิทยาลัยแม่ฟ้าหลวง	อนุกรรมการ
๑๐. ผู้อำนวยการสถาบันนวัตกรรมการเรียนรู้มหาวิทยาลัยแม่ฟ้าหลวง	อนุกรรมการ
๑๑. ผู้อำนวยการสำนักงานจัดการทรัพย์สินและรายได้	อนุกรรมการ
๑๒. หัวหน้าส่วนประชาสัมพันธ์	อนุกรรมการ
๑๓. หัวหน้าส่วนอาคารสถานที่	อนุกรรมการ
๑๔. หัวหน้าหน่วยจัดการสารสนเทศ	อนุกรรมการ
๑๕. หัวหน้าส่วนการเงินและบัญชี	อนุกรรมการ
๑๖. หัวหน้าส่วนพัฒนาความสัมพันธ์ระหว่างประเทศ	อนุกรรมการ
๑๗. หัวหน้าส่วนนโยบายและแผน	อนุกรรมการ
๑๘. อาจารย์ ทันตแพทย์หญิงรพินท์ อบสุวรรณ	อนุกรรมการ
๑๙. นางสาววริวรรณ สุขผล	อนุกรรมการ
๒๐. รองศาสตราจารย์ ทันตแพทย์หญิง ดร.คมขำ พัฒนาภรณ์	อนุกรรมการและเลขานุการ
๒๑. อาจารย์ ทันตแพทย์หญิงธัญวรัตม์ ศรีเมือง	อนุกรรมการและผู้ช่วยเลขานุการ
๒๒. เภสัชกรเอกรัตน์ มีภู	อนุกรรมการและผู้ช่วยเลขานุการ
๒๓. นายนภัสดล เมืองมูล	อนุกรรมการและผู้ช่วยเลขานุการ
๒๔. นางสาวมัลลิกา เกษม	อนุกรรมการและผู้ช่วยเลขานุการ

หน้าที่

๑. วางแผน กำหนดแนวทาง และให้การสนับสนุนการดำเนินงานของคณะอนุกรรมการด้านต่างๆ ที่เกี่ยวข้องกับการจัดประชุมวิชาการฯ

๒. กำหนดรูปแบบพิธีเปิดประชุมวิชาการฯ และจัดหาผู้ดำเนินรายการพิธีเปิด

๓. จัดทำกำหนดการประชุมวิชาการฯ

/๔. กำหนดรูปแบบ...

๔. กำหนดรูปแบบและกำหนดการงานเลี้ยงต้อนรับผู้เข้าร่วมประชุมวิชาการฯ และจัดหาผู้ดำเนินรายการ

๕. จัดหาและประสานงานนักศึกษาช่วยงานของแต่ละฝ่าย

๖. ติดต่อประสานงานกับคณะอนุกรรมการฝ่ายต่าง ๆ และ/หรือหน่วยงานอื่นที่เกี่ยวข้องกับการประชุมวิชาการ

๗ .ดำเนินงานอื่นใดตามที่มหาวิทยาลัยมอบหมาย

๒. คณะอนุกรรมการฝ่ายวิชาการ

๑. รองศาสตราจารย์ ทันตแพทย์หญิง ดร.คมขำ พัฒนาภรณ์	ประธานอนุกรรมการ
๒. อาจารย์ ทันตแพทย์สุมิตร สูอำพัน	รองประธานอนุกรรมการ
๓. ศาสตราจารย์คลินิกเกียรติคุณ ทันตแพทย์หญิง ดร.วรุณี เกิดวงศ์บัณฑิต	อนุกรรมการ
๔. อาจารย์ ทันตแพทย์ ดร.ณรงค์ ลุมพิกานนท์	อนุกรรมการ
๕. ผู้ช่วยศาสตราจารย์ ทันตแพทย์หญิง ดร.วิไลรัตน์ วรภมร	อนุกรรมการ
๖. อาจารย์ ดร.อนุพงศ์ เมฆอุดม	อนุกรรมการ
๗. อาจารย์ ทันตแพทย์มานพ ยศดี	อนุกรรมการ
๘. อาจารย์ ทันตแพทย์หญิงนุชนันท์ เศวตโชติ	อนุกรรมการ
๙. อาจารย์ ทันตแพทย์หญิงธัญวรัตม์ ศรีเมือง	อนุกรรมการ
๑๐. อาจารย์ ทันตแพทย์หญิงพิชญา ชนะกิจเสรี	อนุกรรมการ
๑๑. อาจารย์ ทันตแพทย์รวีโรจน์ รักไทยนิยม	อนุกรรมการ
๑๒. อาจารย์ ทันตแพทย์หญิงวิไล อริยะวุฒิกุล	อนุกรรมการและเลขานุการ
๑๓. นางสาวจารุวรรณ พงษ์นุวัฒน์	อนุกรรมการและผู้ช่วยเลขานุการ
๑๔. นายนภัสดล เมืองมูล	อนุกรรมการและผู้ช่วยเลขานุการ
๑๕. นางสาวเพียงตะวัน วันดี	อนุกรรมการและผู้ช่วยเลขานุการ
๑๖. นางศุทธินี แหลมไธสง	อนุกรรมการและผู้ช่วยเลขานุการ
๑๗. นางสาวหทัยญณัฐ ใจโลกา	อนุกรรมการและผู้ช่วยเลขานุการ

หน้าที่

๑. จัดเตรียมเอกสารประกอบการประชุมวิชาการฯ รายงานสืบเนื่องจากการประชุม (Conference Proceeding) เล่มบทคัดย่อ (Abstract book) และสูจิบัตร

๒. จัดหาผู้ดำเนินรายการสรุปเรื่อง (Chairman) ประจำแต่ละ Session

/๓. รวบรวมไฟล์...

๓. รวบรวมไฟล์นำเสนอของวิทยากร ผู้ทรงคุณวุฒิ และผู้นำเสนอผลงาน
๔. จัดลำดับการนำเสนอผลงานทั้งภาคบรรยายและโปสเตอร์
๕. ออกหนังสือเชิญวิทยากร ผู้ทรงคุณวุฒิ และ/หรือ ผู้ดำเนินรายการสรุปเรื่อง (Chairman)
๖. ติดต่อประสานงานกับคณะอนุกรรมการฝ่ายต่าง ๆ และ/หรือหน่วยงานอื่นที่เกี่ยวข้องกับการประชุมวิชาการ
๗. ดำเนินงานอื่นใดตามที่มหาวิทยาลัยมอบหมาย

๓. คณะอนุกรรมการพิจารณาบทความทางวิชาการ (Editorial Board)

ผู้ทรงคุณวุฒิจากประเทศไทย (Thai Editorial Board)

๑. ศาสตราจารย์คลินิกเกียรติคุณ ทันตแพทย์หญิง ดร.วรุณี เกิดวงศ์บัณฑิต	ประธานอนุกรรมการ
๒. รองศาสตราจารย์ ทันตแพทย์หญิง ดร.คมขำ พัฒนาภรณ์	รองประธานอนุกรรมการ
๓. ศาสตราจารย์ ทันตแพทย์หญิงสมรตรี วิถีพร	อนุกรรมการ
๔. ศาสตราจารย์เชี่ยวชาญพิเศษ ทันตแพทย์ ดร.สุทธิชัย กฤษณะประกรกิจ	อนุกรรมการ
๕. ศาสตราจารย์ ทันตแพทย์ ดร.นพคุณ วงษ์สวรรค์	อนุกรรมการ
๖. ศาสตราจารย์ ทันตแพทย์ นายแพทย์ธงชัย นันทนรานนท์	อนุกรรมการ
๗. ศาสตราจารย์เชี่ยวชาญพิเศษ ทันตแพทย์ ดร.อะนัฆ เอี่ยมอรุณ	อนุกรรมการ
๘. ศาสตราจารย์ ทันตแพทย์ ดร.ธนภูมิ โอสถานนท์	อนุกรรมการ
๙. รองศาสตราจารย์ ทันตแพทย์หญิง ดร.ศิริวิมล ศรีสวัสดิ์	อนุกรรมการ
๑๐. รองศาสตราจารย์ ทันตแพทย์หญิง ดร.ศิริรักษ์ นครชัย	อนุกรรมการ
๑๑. รองศาสตราจารย์ ทันตแพทย์พิสัยศิษฎ์ ชัยจรีนนท์	อนุกรรมการ
๑๒. รองศาสตราจารย์ ทันตแพทย์หญิง ดร.อรนาฏ มาตังคสมบัติ	อนุกรรมการ
๑๓. ผู้ช่วยศาสตราจารย์ ทันตแพทย์หญิง ดร.วิไลรัตน์ วรภมร	อนุกรรมการ
๑๔. ผู้ช่วยศาสตราจารย์ ทันตแพทย์หญิง ดร.จารุมา ศักดิ์ดี	อนุกรรมการ
๑๕. ผู้ช่วยศาสตราจารย์ ทันตแพทย์ ดร.อนุพันธ์ สิทธิโชคชัยวุฒิ	อนุกรรมการ
๑๖. ผู้ช่วยศาสตราจารย์ ทันตแพทย์ ดร.ณัฐพงษ์ กันตรง	อนุกรรมการ

ผู้ทรงคุณวุฒิจากต่างประเทศ (International Editorial Board)

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๑๘. Professor Nabil Samman (Hongkong)	อนุกรรมการ

/๑๙. Professor...

๑๙. Professor Dr.Junfang Xiao (China)	อนุกรรมการ
๒๐. Professor Dr.Delin Xia (China)	อนุกรรมการ
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๒๕. Associate Professor Dr.Russell Gindano II (USA)	อนุกรรมการ
๒๖. Assistant Professor Naseeba Khouja (Saudi Arabia)	อนุกรรมการ
๒๗. Clinical Associate Professor Ana E Evans (USA)	อนุกรรมการ
๒๘. Dr.Ana Maria Chang (USA)	อนุกรรมการ
๒๙. อาจารย์ ทันตแพทย์สุมิตร สูอำพัน	อนุกรรมการและเลขานุการ
๓๐. อาจารย์ ทันตแพทย์รวีโรจน์ รักไทยนิยม	อนุกรรมการและผู้ช่วยเลขานุการ
๓๑. นายนภัสดล เมืองมูล	ผู้ช่วยเลขานุการ

หน้าที่

๑. พิจารณาสรรหาและประสานงานผู้ตรวจอ่าน (Reviewer)
๒. พิจารณาคัดเลือก และตรวจสอบบทความของผู้สมัคร
๓. ดำเนินการแก้ไข (Review) บทคัดย่อสำหรับ Oral และ Poster Presentation รวมทั้ง Proceeding ที่ส่งเข้าร่วมนำเสนอผลงาน
๔. ดำเนินงานอื่นใดตามที่มหาวิทยาลัยมอบหมาย

๔.คณะอนุกรรมการฝ่ายลงทะเบียน ต้อนรับ รับรองวิทยากรและผู้ทรงคุณวุฒิ

๑. อาจารย์ ทันตแพทย์หญิงจิรวรรณ ชาติพิทักษ์	ประธานอนุกรรมการ
๒. อาจารย์ ทันตแพทย์หญิงวิไล อริยะวุฒิกุล	รองประธานอนุกรรมการ
๓. หัวหน้าส่วนพัฒนาความสัมพันธ์ระหว่างประเทศ	อนุกรรมการ
๔. หัวหน้าส่วนประชาสัมพันธ์	อนุกรรมการ
๕. อาจารย์ ทันตแพทย์หญิงอัยยมิญช์ ฉันทศิริพันธุ์	อนุกรรมการ
๖. อาจารย์ ทันตแพทย์เอกพงศ์ เดชธรรม	อนุกรรมการ
๗. อาจารย์ ทันตแพทย์คุณาธิป สุขประเสริฐ	อนุกรรมการ

/๘. อาจารย์...

๘. อาจารย์ ทันตแพทย์ครองพล ชื่นบาน	อนุกรรมการ
๙. อาจารย์ ทันตแพทย์นิติกร อินทพงษ์	อนุกรรมการ
๑๐. อาจารย์ ทันตแพทย์หญิงปาณิศา หอมใย	อนุกรรมการ
๑๑. นางสาวพิรญาณ์ พุทธาศรี	อนุกรรมการ
๑๒. นางสาววารุณี แก้วบุญเรือง	อนุกรรมการ
๑๓. นางสาวพรพิสุทธิ์ ฤศุภร	อนุกรรมการ
๑๔. นางอัจฉรา ชุณห์ช่วงโชติ	อนุกรรมการ
๑๕. นางสาวอรทัย เต็มใจ	อนุกรรมการและเลขานุการ
๑๖. นายพินิจ มุงเมฆ	อนุกรรมการและผู้ช่วยเลขานุการ
๑๗. เภสัชกรเอกรัตน์ มีภู	อนุกรรมการและผู้ช่วยเลขานุการ
๑๘. นายนภัสดล เมืองมูล	อนุกรรมการและผู้ช่วยเลขานุการ
๑๙. นายนพพิชญ์ จันดี	อนุกรรมการและผู้ช่วยเลขานุการ
๒๐. นางสาวเพียงตะวัน วันดี	อนุกรรมการและผู้ช่วยเลขานุการ

หน้าที่

๑. ออกหนังสือเชิญ ผู้สนับสนุน แขกผู้ทรงเกียรติ และหน่วยงานตามที่ได้รับมอบหมาย เข้าร่วมพิธีเปิดการประชุมวิชาการฯ และงานเลี้ยงขอบคุณ

๒. หนังสือเชิญเข้าร่วมงานประชุมฯ โดยไม่ถือเป็นวันลา

๓. จัดเตรียม ของที่ระลึก สำหรับ Invited speakers ผู้ทรงคุณวุฒิ และ/หรือผู้ดำเนินรายการ สรุปเรื่อง (Chairman)

๔. จัดเตรียมรายละเอียดเกี่ยวกับการเดินทางและที่พักของวิทยากรและผู้ทรงคุณวุฒิที่เชิญมาร่วมการประชุมวิชาการฯ

๕. ให้การต้อนรับและอำนวยความสะดวกแก่วิทยากรและผู้ทรงคุณวุฒิที่เชิญมาร่วมการประชุมวิชาการฯ

๖. จัดเตรียมยานพาหนะ ที่พัก เพื่อรับรองวิทยากรและผู้ทรงคุณวุฒิที่เชิญมาร่วมการประชุมวิชาการฯ

๗. ติดต่อประสานงานกับคณะอนุกรรมการฝ่ายต่าง ๆ และ/หรือหน่วยงานอื่นที่เกี่ยวข้องกับการประชุมวิชาการฯ

๘. วางแผน บริหารจัดการรับลงทะเบียนผ่านช่องทางออนไลน์และหน้างาน

/๕. คณะอนุกรรมการ...

๕. คณะอนุกรรมการฝ่ายเว็บไซต์ ประชาสัมพันธ์ และประเมินผล

๑. อาจารย์ ทันตแพทย์มานพ ยศดี	ประธานอนุกรรมการ
๒. อาจารย์ ทันตแพทย์หญิงพัทธ์ธีรา บุญกว้าง	รองประธานอนุกรรมการ
๓. หัวหน้าส่วนประชาสัมพันธ์	อนุกรรมการ
๔. หัวหน้าหน่วยจัดการสารสนเทศ	อนุกรรมการ
๕. อาจารย์ ทันตแพทย์หญิงรวิสรา แสนสิริ	อนุกรรมการ
๖. อาจารย์ ดร.อนุพงศ์ เมฆอุดม	อนุกรรมการ
๗. อาจารย์ ทันตแพทย์หญิงอัยยมิญช์ ฉันทศิริพันธุ์	อนุกรรมการ
๘. อาจารย์ ทันตแพทย์สิรวิชญ์ สุทธาโรจน์	อนุกรรมการ
๙. อานายเกรียงศักดิ์ สมบัตินันทน์	อนุกรรมการ
๑๐. นายธีรศักดิ์ สีสัน	อนุกรรมการ
๑๑. นางสาวจารุวรรณ พงษ์นุวัฒน์	อนุกรรมการ
๑๒. นางสาวเมทินี แซ่ตั้ง	อนุกรรมการ
๑๓. นางสาวพิกุล จันทะศรี	อนุกรรมการ
๑๔. นางศิริลักษณ์ วีราคม	อนุกรรมการ
๑๕. นางสาวพรพิสุทธิ์ ฤศุภร	อนุกรรมการ
๑๖. นางสาวรมณ รวยแสน	อนุกรรมการ
๑๗. นางสาวปวีณ์สุดา นุภาพ	อนุกรรมการ
๑๘. นายจักรพงษ์ วงศ์ใหญ่	อนุกรรมการ
๑๙. นางสาวนฤมล จันทองอนันต์	อนุกรรมการ
๒๐. อาจารย์ ทันตแพทย์หญิงเบญจมาภรณ์ รังษีภาณุรัตน์	อนุกรรมการและเลขานุการ
๒๑. นางสาวเลิศลักษณ์ สงวนสิน	อนุกรรมการและผู้ช่วยเลขานุการ
๒๒. นายนพพิชญ์ จันดี	อนุกรรมการและผู้ช่วยเลขานุการ
๒๓. นางสาวจิตินันท์ กาวี	อนุกรรมการและผู้ช่วยเลขานุการ

หน้าที่

๑. จัดทำเว็บไซต์ประชาสัมพันธ์การจัดการประชุมวิชาการฯ

๒. จัดทำแบบประเมินและสรุปผลผู้เข้าร่วมการประชุมวิชาการ

/๓. ประชาสัมพันธ์...

๓. ประชาสัมพันธ์การจัดประชุมวิชาการฯ สู่สาธารณะโดยสื่อแขนงต่าง ๆ ทั้งภายในและภายนอกมหาวิทยาลัย
๔. จัดทำป้ายประชาสัมพันธ์ ป้ายอื่น ๆ ที่เกี่ยวข้องสำหรับการประชุมวิชาการฯ
๕. จัดทำหนังสือเชิญถึงหน่วยงานที่เกี่ยวข้องเพื่อประชาสัมพันธ์ข้อมูลการประชุมวิชาการฯ
๖. จัดเตรียมเอกสารอื่นที่เกี่ยวข้อง สำหรับผู้ร่วมงาน (ถ้ามี) เช่น ประกาศนียบัตร
๗. จัดเตรียมสื่อ โปสเตอร์ สำหรับการถ่ายทอดสด คอมพิวเตอร์ และโสตทัศนูปกรณ์ (ถ้ามี)
๘. ติดต่อประสานงานกับคณะอนุกรรมการฝ่ายต่าง ๆ และ/หรือหน่วยงานอื่นที่เกี่ยวข้องกับการประชุมวิชาการฯ
๙. ดำเนินงานอื่นใดตามที่มหาวิทยาลัยมอบหมาย

๖. คณะอนุกรรมการฝ่ายโสตทัศนูปกรณ์ สถานที่ ยานพาหนะ และเทคโนโลยี

๑. ผู้อำนวยการศูนย์บริการวิชาการ	ประธานอนุกรรมการ
๒. ผู้อำนวยการสถาบันนวัตกรรมการเรียนรู้มหาวิทยาลัยแม่ฟ้าหลวง	รองประธานอนุกรรมการ
๓. ผู้อำนวยการศูนย์บริการเทคโนโลยีสารสนเทศ	อนุกรรมการ
๔. หัวหน้าส่วนอาคารสถานที่	อนุกรรมการ
๕. นายอกณิษฐ์ สุทธนู	อนุกรรมการ
๖. นายภิญโญ คงมีลาภ	อนุกรรมการ
๗. นายเอกลักษณ์ โพธิศาสตร์	อนุกรรมการ
๘. นายศราวุธ ปัญญาใจ	อนุกรรมการ
๙. นายปกรณ์พัศ แก้วรากมุข	อนุกรรมการ
๑๐. นายสุรินทร์ ปัญญาเพ็ชร	อนุกรรมการ
๑๑. นายวีรยุทธ วงค์สุภา	อนุกรรมการ
๑๒. นายมนตรี อุตระกาศ	อนุกรรมการ
๑๓. นายพนมกร นันติ	อนุกรรมการ
๑๔. นายสมชาติ ใจแก้ว	อนุกรรมการ
๑๕. นายสุวัฒน์ชัย หลวงสุภา	อนุกรรมการ
๑๖. นายพันธ์พงศ์ กองตุ้ย	อนุกรรมการ
๑๗. นายธณัติ ดวงสวัสดิ์	อนุกรรมการ
๑๘. นายสุรศักดิ์ ใจจุมปา	อนุกรรมการ

/๑๙. นาย...

๑๙. นายศิวกร ทองดี	อนุกรรมการ
๒๐. นายอัฐกร แสนศรี	อนุกรรมการ
๒๑. นายจิตวัต อินต๊ะมูล	อนุกรรมการ
๒๒. นายจรูญรัตน์ ลือสุวรรณ์	อนุกรรมการ
๒๓. นายอิทธิ คำอ้อย	อนุกรรมการ
๒๔. นายวรวิทย์ คำวัง	อนุกรรมการ
๒๕. นายเทพพิทักษ์ สมนา	อนุกรรมการ
๒๖. อาจารย์ ทันตแพทย์พรรษกร แสงแก้ว	อนุกรรมการ
๒๗. อาจารย์ ทันตแพทย์ปรัชฐกร ศิริอรุณรัตน์	อนุกรรมการ
๒๘. อาจารย์ ทันตแพทย์ธัญพงศ์ มาไพศาลสิน	อนุกรรมการ
๒๙. อาจารย์ ทันตแพทย์อัจฉริยะ วีรนนท์บุรภัทร	อนุกรรมการ
๓๐. อาจารย์ ทันตแพทย์คุณาธิป สุขประเสริฐ	อนุกรรมการ
๓๑. เภสัชกรเอกรัตน์ มีภู	อนุกรรมการและเลขานุการ
๓๒. นายธีรศักดิ์ สีสัน	อนุกรรมการและผู้ช่วยเลขานุการ
๓๓. นางสาวจิตินันท์ กาวี	อนุกรรมการและผู้ช่วยเลขานุการ
๓๔. นางศุทธินี แหลมไธสง	อนุกรรมการและผู้ช่วยเลขานุการ
๓๕. นางสาวหทัยญณัฐ ใจโลกา	อนุกรรมการและผู้ช่วยเลขานุการ
๓๖. นายเกรียงศักดิ์ สมบัตินันทน์	อนุกรรมการและผู้ช่วยเลขานุการ
๓๗. นายคทายุทธ ทาแกง	อนุกรรมการและผู้ช่วยเลขานุการ
๓๘. นางสาวเพียงตะวัน วันดี	อนุกรรมการและผู้ช่วยเลขานุการ

หน้าที่

๑. จัดเตรียมและให้การสนับสนุนเครื่องมือถ่ายทอดสด คอมพิวเตอร์ และโสตทัศนูปกรณ์

๒. จัดเตรียมสถานที่ อุปกรณ์สนับสนุนการประชุมวิชาการฯ อาทิ พิธีเปิด ห้องประชุมต่างๆ และห้องแสดงโปสเตอร์

๓. ดำเนินการถ่ายทอดการประชุมวิชาการฯ ผ่านช่องทางออนไลน์และอื่นๆ (ถ้ามี)

๔. จัดเตรียมยานพาหนะสำหรับรับส่งผู้เข้าร่วมการประชุมวิชาการฯ และภารกิจอื่นในส่วนที่เกี่ยวข้อง

/๕. จัดระบบรักษา...

๕. จัดระบบรักษาความปลอดภัย และจัดการจราจรร่วมกับหน่วยงานที่เกี่ยวข้อง

๖. ติดต่อประสานงานกับคณะอนุกรรมการฝ่ายต่าง ๆ และ/หรือหน่วยงานอื่นที่เกี่ยวข้องกับการประชุมวิชาการฯ

๗. ดำเนินงานอื่นใดตามที่มหาวิทยาลัยมอบหมาย

๗. คณะอนุกรรมการฝ่ายสวัสดิการ

๑. คณบดีสำนักวิชาทันตแพทยศาสตร์	ที่ปรึกษา
๒. คณบดีสำนักวิชาการจัดการ	ที่ปรึกษา
๓. ผู้ช่วยศาสตราจารย์สุกัล กฤตลักษณ์วงศ์ ผู้ช่วยอธิการบดี รักษาการแทนหัวหน้าส่วนพัฒนานักศึกษา	ที่ปรึกษา
๔. อาจารย์ ดร.ณัฐวุฒิ ยอดสุวรรณ ผู้อำนวยการศูนย์บริการวิชาการ	ที่ปรึกษา
๕. อาจารย์ ทันตแพทย์หญิงรพินท์ อบสุวรรณ	ประธานอนุกรรมการ
๖. ผู้อำนวยการสำนักงานจัดการทรัพย์สินและรายได้	รองประธานอนุกรรมการ
๗. หัวหน้าส่วนพัฒนาความสัมพันธ์ระหว่างประเทศ	อนุกรรมการ
๘. หัวหน้าส่วนอาคารสถานที่	อนุกรรมการ
๙. อาจารย์ ทันตแพทย์มานพ ยศดี	อนุกรรมการ
๑๐. อาจารย์ ทันตแพทย์ครองพล ชื่นบาน	อนุกรรมการ
๑๑. อาจารย์ ทันตแพทย์หญิง เบญจมาภรณ์ รังษีภาณุรัตน์	อนุกรรมการ
๑๒. อาจารย์ ทันตแพทย์สิรวิชญ์ สุทธาโรจน์	อนุกรรมการ
๑๓. อาจารย์ ทันตแพทย์หญิงวิไล อริยะวุฒิกุล	อนุกรรมการ
๑๔. อาจารย์ ทันตแพทย์ปรัชฐกร ศิริอรุณรัตน์	อนุกรรมการ
๑๕. อาจารย์ ทันตแพทย์หญิงพัทธ์ธีรา บุญกว้าง	อนุกรรมการ
๑๖. นายธีรศักดิ์ ทะจันทร์	อนุกรรมการ
๑๗. นางสาวชัญญา ตีรณวัฒนากูล	อนุกรรมการ
๑๘. นางสาวธมน ตรีเหรา	อนุกรรมการ
๑๙. นางสาวเสาวนีย์ เชื้อปาละ	อนุกรรมการ
๒๐. อาจารย์ ทันตแพทย์อัจฉริยะ วีรนนท์บุรภัทร	อนุกรรมการและเลขานุการ
๒๑. นายนพพิชญ์ จันดี	อนุกรรมการและผู้ช่วยเลขานุการ

/๒๒. นางสาว...

๒๒. นางสาวจิตินันท์ กาวี	อนุกรรมการและผู้ช่วยเลขานุการ
๒๓. นางสาวพิกุล จันทะศรี	อนุกรรมการและผู้ช่วยเลขานุการ

หน้าที่

๑. จัดเตรียมอาหาร อาหารว่างและเครื่องดื่ม
๒. จัดเตรียมสถานที่สำหรับรับประทานอาหารเครื่องดื่ม
๓. จัดงานเลี้ยงต้อนรับผู้เข้าร่วมประชุมวิชาการฯ
๔. ติดต่อประสานงานกับคณะอนุกรรมการฝ่ายต่าง ๆ และ/หรือหน่วยงานอื่นที่เกี่ยวข้องกับการประชุมวิชาการฯ
๕. ดำเนินงานอื่นใดตามที่มหาวิทยาลัยมอบหมาย

๘. คณะอนุกรรมการฝ่ายจัดหาทุนสนับสนุนการประชุมวิชาการฯ

๑. คณบดีสำนักวิชาทันตแพทยศาสตร์	ประธานอนุกรรมการ
๒. อาจารย์ ทันตแพทย์พิศักดิ์ องค์ศิริมงคล	รองประธานอนุกรรมการ
๓. อาจารย์ ทันตแพทย์ ดร.ณรงค์ ลุมพิกานนท์	อนุกรรมการ
๔. อาจารย์ ทันตแพทย์หญิงรพินท์ อบสุวรรณ	อนุกรรมการ
๕. ทันตแพทย์ปิยะณัฐ สินธุ์ญา	อนุกรรมการ
๖. เภสัชกรเอกรัตน์ มีภู	อนุกรรมการ
๗. นางสาวพิกุล จันทะศรี	อนุกรรมการและเลขานุการ
๘. นางสาวอรทัย เต็มใจ	อนุกรรมการและผู้ช่วยเลขานุการ
๙. นางสาวจารุวรรณ พงษ์นุวัฒน์	อนุกรรมการและผู้ช่วยเลขานุการ

หน้าที่

๑. ติดต่อแหล่งทุนสนับสนุนจากภาคเอกชนเพื่อขอรับการสนับสนุนงบประมาณในการจัดประชุมวิชาการฯ
๒. ดูแลพื้นที่การจัดแสดงนิทรรศการหรือการแสดงสินค้าของห้างร้าน/หน่วยงานภายนอก
๓. จัดเตรียมบูทสำหรับหน่วยงานภาครัฐและเอกชนที่จัดแสดงนิทรรศการ
๔. ติดต่อประสานงานกับคณะอนุกรรมการฝ่ายต่าง ๆ และ/หรือหน่วยงานอื่นที่เกี่ยวข้องกับการประชุมวิชาการฯ

/๕. ดำเนินงาน...

๕. ดำเนินงานอื่นใดตามที่มหาวิทยาลัยมอบหมาย

๙. คณะอนุกรรมการฝ่ายการเงินและงบประมาณ

๑. คณบดีสำนักวิชาทันตแพทยศาสตร์	ประธานอนุกรรมการ
๒. อาจารย์ ทันตแพทย์พิศักดิ์ องค์ศิริมงคล	รองประธานอนุกรรมการ
๓. หัวหน้าส่วนการเงินและบัญชี	อนุกรรมการ
๔. ศาสตราจารย์คลินิกเกียรติคุณ ทันตแพทย์หญิง ดร.วรุณี เกิดวงศ์บัณฑิต	อนุกรรมการ
๕. อาจารย์ ทันตแพทย์ ดร.ณรงค์ ลุมพิกานนท์	อนุกรรมการ
๖. อาจารย์ ทันตแพทย์หญิงจิรวรรณ ชาติพิทักษ์	อนุกรรมการ
๗. เภสัชกรเอกรัตน์ มีภู	อนุกรรมการ
๘. นางสาวผ่องพรรณ นะติกา	อนุกรรมการ
๙. นางสาวพัชรินทร์ ศรีใจ	อนุกรรมการ
๑๐. นางศิริลักษณ์ วีราคม	อนุกรรมการและเลขานุการ
๑๑. นางสาวเมทินี แซ่ตั้ง	อนุกรรมการและผู้ช่วยเลขานุการ
๑๒. นางสาวเพียงตะวัน วันดี	อนุกรรมการและผู้ช่วยเลขานุการ

หน้าที่

๑. จัดทำคำของบประมาณเพื่อเสนอต่อมหาวิทยาลัยหรือแหล่งทุน

๒. ประสานงานและรวบรวมงบประมาณจากอนุกรรมการฝ่ายต่าง ๆ และขออนุมัติเบิกจ่าย

๓. จัดทำรายงานสรุปการใช้จ่ายการประชุมวิชาการฯ

๔. ดำเนินงานอื่นใดตามที่มหาวิทยาลัยมอบหมาย

ทั้งนี้ ตั้งแต่บัดนี้เป็นต้นไป จนเสร็จสิ้นกิจกรรม

สั่ง ณ วันที่ ๓ สิงหาคม พ.ศ. ๒๕๖๕

(รองศาสตราจารย์ ดร.ชยาพร วัฒนศิริ)
อธิการบดีมหาวิทยาลัยแม่ฟ้าหลวง

The 19th International Scientific Conference of the Dental Faculty Consortium of Thailand (DFCT 2022)

"Novel Challenges in Dental Practice and Research"

November 2-4, 2022

November 2, 2022

Pradoo Dang Auditorium I, Sampao Choosri Building (E4)	
08.00-09.00 A.M.	Registration
09.00-09.50 A.M.	**MicroRNA-Based Gene Therapy for Oral and Craniofacial Diseases** **Keynote Speaker**: Professor Dr. Liu Hong, University of Iowa College of Dentistry, Iowa City, Iowa, USA
09.50-10.20 AM.	Open Ceremony
10.20-10.30 A.M.	Coffee break
10.30-11.20 A.M.	**Keystone Pathogen Hypothesis: an interplay of *Porphyromonas gingivalis* with a local Innate Immunity of the Periodontium** **Keynote Speaker**: Asst. Prof. Dr. Nutthapong Kantrong, Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand
11.20-12.00 A.M.	**ADAM9: A Promigratory Molecule in Oral Cancer** **Keynote Speaker**: Professor Dr. Suttichai Krisanaprakornkit, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand
12.00 A.M. -01.00 P.M.	Lunch

Conference Room 1 (517), Conference Room 2 (516), Conference Room 3 (515)	
01.00-04.00 P.M.	**Oral Presentation & Competition**

Exhibition Room (518)	
01.00-05.00 P.M.	**Poster Presentation**

Pradoo Dang Auditorium I	
04.00-05.00 P.M.	**DFCT Committee Annual Meeting for Thai Dental School Administrative Faculty**

November 3, 2022

Pradoo Dang Auditorium I, Sampao Choosri Building (E4)	
09.00-09.50 A.M.	**Molecular Biocompatibility - from a Theory to Clinical Applications in Bone and Dental Pulp Tissue Engineering** **Keynote Speaker**: Professor Dr. Laisheng Lee Chou, Boston University Henry M. Goldman School of Dental Medicine, Boston, Massachusetts, USA
09.50-10.30 A.M.	**Regenerative Endodontics: What Does the Future Hold?** **Keynote Speaker**: Professor Dr. Avina Paranjpe, School of Dentistry, University of Washington, Seattle, Washington, USA
10.30-10.40 A.M.	Coffee break
Doitung room, Wanchai Sirichana Building (AD1)	
04.30-06.00 P.M.	DFCT Committee Monthly Meeting (DFCT Executive Board Member only)
Indoor Stadium and Sports Centre (Chalermprakiat Building)	
06.00-09.00 P.M.	Dinner and Award Announcement

Conference room 1 (517)	
10.40-11.20 A.M.	**The Role of PRMT4 in Murine Posterior Frontal Suture Closure** **Speaker**: Dr. Nicha Ungvijanpunya, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
11.20-12.00 A.M.	**MRGPRX2 Signaling on Mast Cell-mediated Neuroimmune Interaction** **Speaker**: Dr. Chalatip Chompunud Na Ayudhya, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand
12.00 A.M.-01.00 P.M.	Lunch
01.00-04.00 P.M.	**Oral Presentation**

Conference room 2 (516)	
10.40-11.20 A.M.	**Research and Development of Fluoride Varnish for Geriatric Dentistry** **Speaker**: Assoc. Prof. Dr. Waleerat Sukarawan, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
11.20-12.00 A.M.	**Oral Health and Nutrition, an Update** **Speaker**: Assoc. Prof. Dr. Dunyaporn Trachootham, Institute of Nutrition, Mahidol University, Bangkok, Thailand
12.00 A.M.-01.00 P.M.	Lunch
01.00-04.00 P.M.	**Oral Presentation**

November 3, 2022

Conference room 3 (515)	
01.00-04.00 P.M.	**Oral Presentation & Competition**

Exhibition Room (518)	
01.00-05.00 P.M.	**Poster Presentation**

Conference room 4 (514)	
09.00-12.00 A.M.	**Dental Public Health Education Seminar-Thai Language (on site only)** **Chairman**: Ass. Prof. Dr. Songvuth Tuongnatanaphan
12.00 A.M.-01.00 P.M.	Lunch
01.00-04.00 P.M.	**Panel Discussion: Dentistry at the Crossroads (on site, zoom meeting)** **Chairman**: Ass. Prof. Dr. Songvuth Tuongnatanaphan
01.00-01.30 P.M.	Dentistry at the Crossroads: Disruption and Change **Speaker**: Dr. Krissada Raungarreerat
01.30-03.00 P.M.	Panel Discussion: Faculty of Dentistry at the Crossroads **Speaker**: Dr. Suchit Poolthong Dr. Narumanas Korwanick Mr. Sansern Namphom **Moderator**: Dr. Paisan Kangvonkit
03.00-04.00 P.M.	Panel Discussion: Community Dentistry at the Crossroads **Speaker**: Dr. Wararat Jaichuen Dr. Wirat Eungpoonsawat Dr. Waranuch Pitiphat **Moderator**: Dr. Pisak Ongsirimongkol
04.00-04.30 P.M.	Open Discussion

November 4, 2022

Pradoo Dang Auditorium I, Sampao Choosri Building (E4)	
09.00-10.00 A.M.	**Dental Education in the Post-COVID-19 Era** **Keynote Speaker**: Asst. Prof. Dr. Supachai Chuenjitwongsa, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
End of online broadcasting	
10.00-10.10 A.M.	Coffee break
10.10-11.45 A.M.	Dental Education Seminar I (Thai Language) Group Discussions of The Thai Dental Faculty Well-Being Guideline -Facilities and Atmosphere -Curriculum Development and Assessment -Physical and Psychological Care Protocols
11.45-12.00 A.M.	The Thai Dental Faculty Well-being Guideline MOU Signing Ceremony
12.00 A.M.-01.00 P.M.	Lunch
01.00-02.30 P.M.	Dental Education Seminar II (Thai Language) “Sharing Best Practices” session -Facilities and Atmosphere -Curriculum Development and Assessment
02.30-02.45 P.M.	Coffee break
02.45-04.00 P.M.	Dental Education Seminar III (Thai Language) -Physical and Psychological Care Protocols

Scientific Program of Oral Presentation
November 2, 2022

Conference Room 1 (517) Oral Presentation 1:00–4:00 P.M. Thailand local time, (GMT+7); Nov 2, 2022 Chairman: Professor Dr. Pornchai Jansisyanont Co-Chairman: Assoc. Prof. Dr. Samroeng In-glam		
01:00-01:15	OA-00002	**ELANE Mutation Altering Dental Pulp Cell Biomolecular Processes** ***Kanokwan SRIWATTANAPONG,***[1] *Noppadol Sa-Ard-Iam,*[2] *Lawan Boonprakong,*[3] *and Thantrira Porntaveetus*[1] [1]*Center of Excellence in Genomics and Precision Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330, Thailand* [2]*Center of Excellence in Periodontal Disease and Dental Implant, Immunology Research Center, Faculty of Dentistry, Chulalongkorn University, Bangkok, 10330, Thailand* [3]*Oral Biology Research Center, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330, Thailand*
01:15-01:30	OA-00005	**Cannabidiol Enhances Differentiation and Biomineralization of Primary Human Osteoblasts via the Canonical Pathway** ***Anupong MAKEUDOM,***[1] *Natthaorn THANAI-NOPPARAT,*[2] *Chayarop SUPANCHART,*[3] *Pattanin MONTREEKACHON,*[2] *and Suttichai KRISANAPRAKORNKIT*[4] [1]*School of Dentistry, Mae Fah Luang University, Chiang Rai, 57100* [2]*Department of Restorative Dentistry and Periodontology, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200* [3]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200* [4]*Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200*
01:30-01:45	OA-00007	**The Study of PD-L1 and RPS6 Expression in Oral Epithelial Dysplasia and Squamous Cell Carcinoma** ***Jaruwat Hanroongsri***[1] *and Risa Chaisuparat*[2] [1]*Department of Oral Pathology, Faculty of Dentistry, Chulalongkorn University, Pathumwan, Bangkok, Thailand* [2]*Exocrine Gland Biology and Regeneration Research Group, Faculty of Dentistry, Chulalongkorn University, Pathumwan, Bangkok, Thailand*
01:45-02:00	OA-00019	**Extracellular pH Modulates the Second Messenger Concentrations in *Streptococcus mutans*** ***Kasidid RUKSAKIET,***[1] *Balázs STERCZ,*[2] *Gergő TÓTH,*[3] *Pongsiri JAIKUMPUN,*[1] *Orsolya DOBAY,*[2] *Péter HORVÁTH,*[3] *Ákos ZSEMBERY,*[4] *and Z. M. LOHINAI*[5] [1]*Department of Restorative Dentistry, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand* [2]*Institute of Medical Microbiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary* [3]*Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Semmelweis University, Budapest, Hungary* [4]*Department of Oral Biology, Faculty of Dentistry, Semmelweis University, Budapest, Hungary* [5]*Department of Conservative Dentistry, Faculty of Dentistry, Semmelweis University, Budapest, Hungary*
02:00-02:15	OA-00020	**Substrate Stiffness Regulates Proinflammatory Responses in Gingival Fibroblasts** ***Watcharaphol TISKRATOK,***[1,2] *Masahiro YAMADA,*[2] *Jun WATANABE,*[2] *and Hiroshi EGUSA*[2] [1]*School of Geriatric Oral Health, Institute of Dentistry, Suranaree University of Technology, Nakhon Ratchasima, Thailand* [2]*Division of Molecular and Regenerative of Prosthodontics, Tohoku University Graduate of Dentistry, Sendai, Miyagi, Japan*
02:15-02:30	Coffee break	

02:30-02:45	OA-00021	**RANKL-Polarized Macrophages in Bone Healing: *In Vitro* and *In Vivo* Studies** ***Apichai YAVIRACH***[1] *and Cecilia M. GIACHELLI*[2] [1]*Department of Prosthodontics, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand* [2]*Department of Bioengineering, University of Washington, Seattle, USA*
02:45-03:00	OJ-00001	**Protein Expression after Gingival Injection of mRNA Encoding Platelet-derived Growth Factors-BB in Ligature-induced Periodontitis Model in Rats** ***Pimphorn MEEKHANTONG***,[1,2] *Wichaya WISITRASAMEEWONG,*[1,3,4] *Noppadol SA-ARD-IAM,*[4] *Theeraphat CHANAMUANGKON,*[5] *Somchai YODSANGA,*[6] *Pimprapa RERKYEN,*[4] *and Rangsini MAHANONDA*[1,3,4] [1]*Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* [2]*School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand* [3]*Center of Excellence in Periodontal Disease and Dental Implant, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* [4]*Immunology Research Center, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* [5]*Biomaterial Testing Center, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* [6]*Department of Oral Pathology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand*
03:00-03:15	OJ-00003	**Expression of CLLD7 and CHC1L Proteins in Oral Potentially Malignant Disorders in a Group of Thais: A Preliminary Study** ***Sunisa Suchitanant***,[1] *Siribang-on Piboonniyom Khovidhunkit,*[1] *Rachai Juengsomjit,*[2] *Ounruean Meesakul,*[2] *Sopee Poomsawat,*[2] *Bishwa Prakash Bhattarai,*[3] *and Boworn Klongnoi*[4] [1]*Department of Advanced General Dentistry, Faculty of Dentistry, Mahidol University, Bangkok, Thailand* [2]*Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand* [3]*Walailak University International College of Dentistry, Walailak University, Bangkok, Thailand* [4]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Mahidol University, Bangkok, Thailand epartment of Periodontology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand*
03:15-03:30	OM-00016	**An *Oroxylum Indicum* Extract, A Potential Biomaterial for the Treatment of Periimplantitis** *Jarutai PRAPAIPITTAYAKHUN,*[1] *Supakorn Boonyuen,*[2,4] *Alvin Lim Teik Zheng,*[2] *Komsan Apinyauppatham,*[3,4] *and Premjit Arpornmaeklong*[3,4] [1]*Master of Science Program in Dental Implantology, Faculty of Dentistry* [2]*Department of Chemistry, Faculty of Science and Technology* [3]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry* [4]*Thammasat University Research Unit in Tissue Engineering and Implant Dentistry, Thammasat University-Rangsit campus, Khlong Luang, Pathum Thani 12121, Thailand*
03.30-03.45	OM-00022	**Anti-FimA Antiserum Increases *Porphyromonas gingivalis* Invasion into H357 Cells** *Jantipa JOBSRI,*[1] *Nattachai SAIWARIN,*[1] *Thanit PRASITSAK,*[1] *Warayut CHOTPRAKAIKIAT,*[1] *Niratcha CHAISOMBOON,*[1] *Kusuma JAMDEE,*[1] *Sanya CHIAMSUK,*[1] *and Sorapong WONGNOI*[1] [1]*Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand*
03.45-04.00	OM-00023	**Evaluation of Different Scaffolds for Bone Regeneration in Rat Calvarial Bone Defects** *Jeerawit SIRAKITTIWORAPONG,*[1] *Rangsini MAHANONDA,*[1,2,3] *Theeraphat SURISAENG,*[1] *Noppadol SA-ARD-IAM,*[2,3] *Theeraphat CHANAMUANGKON,*[4] *Somchai YODSANGA,*[5] *Pimprapa RERKYEN*[2,3] *and Wichaya WISITRASAMEEWONG*[1,2,3] [1]*Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* [2]*Center of Excellence in Periodontal Disease and Dental Implant, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* [3]*Immunology Research Center, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* [4]*Biomaterial Testing Center, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* [5]*Department of Oral Pathology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand*

Conference Room 2 (516) Oral Presentation 1:00–4:00 P.M. Thailand local time, (GMT+7); Nov 2, 2022 Chairman: Assoc. Prof. Dr. Waranuch Pitiphat Co-Chairman: Assoc. Prof. Dr. Araya Phonghanyudh		
01:00-01:15	OA-00017	**Empathy Level Towards Patients Among Thai Dental Students** ***Palinee DETSOMBOONRAT***,[1] *Sudthida THEPPANICH,[2] Suttida BANYEN,[2] Sansern HONGVIPHAT,[2] and Anupap SOMBOONSAVATDEE[3]* [1]*Faculty of Dentistry, Chulalongkorn University, Thailand* [2]*Department of Statistics, Chulalongkorn Business School, Chulalongkorn University, Thailand*
01:15-01:30	OA-00022	**Discrepancies in Parent-Child Reports on Children's Oral Health-Related Quality of Life** ***Wilai ARIYAVUTIKUL***[1] *and Varangkanar Jirarattanasopha*[2] [1]*School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand* [2]*Faculty of Dentistry, Mahidol University, Bangkok, Thailand*
01:30-01:45	OJ-00004	**The Efficiency of Reducing the Dispersion Aerosols by using Various Types of Intraoral and Extraoral Suction** ***Mayurach PIPATPHATSAKORN***,[1] *Anuphan Sittichokechaiwut,[2] and Pornsuda Norchai*[2] [1]*Department of Restorative Dentistry, Faculty of Dentistry, Naresuan University, Muang, Phitsanulok, 65000* [2]*Department of Preventive Dentistry, Faculty of Dentistry, Naresuan University, Muang, Phitsanulok, 65000*
01:45-02:00	OJ-00006	**Creating a Gingival Color Database among Thai Samples by Using Digital Images Processing** ***Suriyan THAMMARAT***,[1] *Anuphan Sittichokechaiwut,[2] Suttipalin Suwannakul,[3] and Sasitharee Nathamtong*[4] *Department of Periodontology, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand*
02:00-02:15	OM-00024	**Anti-Inflammatory Effect of *Gynura Procumbens* Extracts on *Aggregatibacter Actinomycetemcomitans* LPS-Stimulated Macrophage Cells** *Thanatchaya WEERAWUTHIKRAI*[1] *Prima BURANASIN,[2] and Chuencheewit THONGSIRI*[2] [1]*Graduate School, Faculty of Dentistry, Srinakharinwirot University, Bangkok, Thailand* [2]*Department of Conservative Dentistry and Prosthodontics, Faculty of Dentistry, Srinakharinwirot University, Bangkok, Thailand*
02:15-02:30	Coffee break	
02:30-02:45	OM-00010	**Expected Barriers in Providing Geriatric Dental Care in Thailand: Questionnaire Validation Study** *Khai Quang DAO,[1] Jutarat VIBULPHOL,[2] and Orapin KOMIN*[1] [1]*Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* [2]*Faculty of Education, Chulalongkorn University, Bangkok, Thailand*
02:45-03:00	OM-00020	**Oral Health Care service in Nursing Homes in Bangkok** Puntawee BARAMEEPIPAT,[1] Nareudee LIMPUANGTHIP,[1] and Orapin KOMIN[1] [1]Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
03:00-03:15	OM-00026	**Dental Services Model for Older Adults in Remote Area: A Qualitative Study** *Thongpak NINLAPUN,[1,3] Narumanas KORWANICH,[2] and Kanyarat KORWANICH*[2] [1]*MS student in Geriatric Dentistry Program, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand* [2]*Department of Family and Community Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand. 3Pangmapha Hospital, Mae Hong Son Province*
03:15-03:30	OM-00028	**Patients' Perception of Dental Treatment during COVID-19 Pandemic: Qualitative Interview** *Paratcha PINGSUTHIWONG,[1] Keskanya SUBBALEKHA,[1] and Paswach WIRIYAKIJJA*[1] [1]*Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand*

03.30-03.45	OM-00030	**Need Analysis for Thai Dental Assistants in Professional Development** *Kasekarn KASEVAYUTH,[1,2] Kaittiyos KULDEJCHAICHAN,[1] and Arb-aroon LERTKHACHONSUK[3]* *[1]Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand* *[2]Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* *[3]Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*
03.45-04.00	OM-00031	**Counseling Method Increasing Knowledge, Motivation and Behavior in Mothers of Preschool Children** *Yudha Rahina,[1] Pande P. Januraga,[2] I Made A. Wirawan,[2] Dewa M. Wedagama,[1] Pudak Elang[1]* *[1]Faculty of Dentistry, Mahasaraswati Denpasar University, Denpasar, Indonesia* *[2]Medical School, Udayana University, Denpasar, Indonesia*
Conference Room 3 (515) **Oral Presentation 1:00–3:45 P.M. Thailand local time, (GMT+7); Nov 2, 2022** **Chairman: Ass. Prof. Dr. Narumanas Korwanich** **Co-Chairman: Professor Dr. Pasutha Thunyakitpisal**		
01:00-01:15	OM-00009	**Move to Competition**
01:15-01:30	OM-00011	**Translation and Cross-cultural Adaptation of the Thai Version of OHIP-TMDs** *Ronlada PORNTIRIT[1] and Chayanit CHAWEEWANNAKORN[1]* *[1]Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand*
01:30-01:45	Preparation for Competition Session (Postgraduate)	
01:45-02:00	OC-00001	**Remineralization of Calcium Silicate Cement in Artificial Caries Affected Dentine** ***Onwara Akkaratham***[1] *and Sirichan Chiaraputt[1]* *[1]Department of Conservative Dentistry and Prosthodontics, Faculty of Dentistry, Srinakharinwirot University, Bangkok, Thailand*
02:00-02:15	OC-00003	**Effects of compressive stress combined with mechanical vibration on osteoclastogenesis in RAW 264.7 cells** ***Boontida Changkhaokham***[1] *[1]Orthodontic Section, Department of Preventive Dentistry, Faculty of Dentistry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*
02:15-02:30	OC-00004	**Effects of Calcium Carbonate Microcapsules on Property of the Thermosensitive Hydrogel** ***Natthaporn JAIMAN***,[1] *Supakorn Boonyuen,[2,4] Asira Fuongfuchat,[5] Komsan Apinyauppatham,[3,4] and Premjit Arpornmaeklong[3,4]* *[1]Master of Science Program in Oral and Maxillofacial Surgery, Faculty of Dentistry,* *[2]Department of Chemistry, Faculty of Science and Technology* *[3]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry,* *[4]Thammasat University Research Unit in Tissue Engineering and Implant Dentistry, Thammasat University Rangsit campus, Pathum Thani 12121, Thailand and* *[5]National Metal and Materials Technology Center (MTEC), Pathum Thani 12121, Thailand*
02:30-02:45	OC-00005	**Identification of Contact Between Third Molar and Mandibular Canal by Deep Learning** ***Dhanaporn Papasratorn***,[1] *Suchaya Pornprasertsuk-Damrongsri,[2] Suraphong Yuma,[3] and Warangkana Weerawanich[2]* *[1]Graduate student in Master of Science Program in Dentistry, Major in Oral and Maxillofacial Radiology, Faculty of Dentistry, Mahidol University* *[2]Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Mahidol University* *[3]Department of Physics, Faculty of Science, Mahidol University*

02:45-03:00	OC-00006	**Periostin-Integrin Interaction Regulates Force-induced TGF-β1 and α-SMA Expression by Periodontal Ligament Cells** ***Daneeya Na Nan***,[1] *Nuttha Klinkumhom,[1,2] Vincent Everts,[2,3] and Thanaphum Osathanon,[2,4] and Prasit Pavasant[1,2]* *[1]Center of Excellence in Regenerative Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* *[2]Department of Anatomy, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* *[3]Department of Oral Cell Biology, Faculty of Dentistry, the University of Amsterdam and Vrije Universiteit, Amsterdam, The Netherlands* *[4]Dental Stem Cell Biology Research Unit, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand*
03:00-03:15	OC-00009	**Satisfaction Level of Healthy Subjects on Mucoadhesive Film containing *Acmella oleracea* Extract** ***Sareeya RUNGCHAMRAT***,[1] *Kemporn Kitsahawong,[1] Patimaporn Pungchanchaikul,[1] Jomjai Peerapattana,[2] and Natsajee Nualkeaw[2]* *[1]Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand* *[2]Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand*
03:15-03:30	OC-00012	**Withdrawn**
03.30-03.45	OC-00013	**Effect of Orthodontic Loading Magnitude on the Periodontal Ligament Thickness: A Preliminary Study** ***Patsara Chatvichai***, *Boonsiva Suzuki, and Eduardo Yugo Suzuki* *Department of Orthodontics, Faculty of Dentistry, Bangkokthonburi University, Bangkok, Thailand*

Scientific Program of Oral Presentation
November 3, 2022

Conference Room 1 (517) Oral Presentation 1:00–4:00 P.M. Thailand local time, (GMT+7); Nov 3, 2022 Chairman: Assoc. Prof. Dr. Sirichai Kiattavorncharoen Co-Chairman: Assoc. Prof. Dr. Anuphan Sittichokechaiwut		
01:00-01:15	OA-00008	**Reproducibility of Automated Deep Learning Based Localization of Mandibular Canal on CBCT** ***DDS Jorma Järnstedt,***[1] *MSc [Tech.] Jaakko Sahlsten,*[2] *MSc [Tech.], Joel Jaskari,*[2] *DPhil Kimmo Kaski,*[2,4] *DDS MSc Sangsom Prapayasotok,*[3] *and DDS, MSc, PhD Sakarat Nalampang*[3] [1]*Medical Imaging Centre, Department of Radiology Tampere University Hospital, Teiskontie 35, 33520 Tampere, Finland* [2]*Aalto University School of Science, Maarintie 8, 02150 Aalto, Finland* [3]*Division of Oral and Maxillofacial Radiology, Faculty of Dentistry, Chiang Mai University, Suthep Rd., T. Suthep, A. Muang, Chiang Mai, Thailand* [4]*Alan Turing Institute, British Library, 96 Euston Rd, London NW1 2DB, UK*
01:15-01:30	OA-00010	**The Efficacy of Drinking Green Tea on Bone Healing Following Surgical Removal of Impacted Lower Third Molars: A Radiographic Evaluation** ***Wiweko S****, Sricharoenchai P, Tantiwannarath S, Kaboosaya B* *Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chulalongkorn University*
01:30-01:45	OA-00016	**The Development of New Appropriate Horizontal X-Ray Beam Angulation Image Receptor-Holding Instruments** ***Peerapong Wamasing,***[1] *Hiroshi Watanabe,*[2] *Yumi Tsuchida,*[3] *Naoto Ohbayashi,*[2] *Tetsuya Suzuki,*[3] *and Tohru Kurabayashi*[2] [1]*Department of Oral Diagnosis, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand* [2]*Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Tokyo Medical and Dental University, Tokyo, Japan* [3]*Department of Oral Prosthetic Engineering, Faculty of Dentistry,Tokyo Medical and Dental University, Tokyo, Japan*
01:45-02:00	OA-00018	**Morphological Analysis of Cementoenamel Junction Types in Premolars** ***Tran Quoc Ninh,*** *Nguyen Truong Khang, Ho Van Anh, Nguyen Cao Ky An, Le Nguyen Hai Duy, Huynh Thi Ngoc Thoai, Phan Ba Loc, Do Thi Thao.* *Faculty of Odonto – Stomatology, Can Tho University of Medicine and Pharmacy, Viet Nam*
02:00-02:15	OJ-00002	**Sensitivity of Brux Checker® in Grinding Bruxer** ***Donlatham Prommasen,***[1] *Namrath Chatchaiyan,*[2,*] *and Somsak Mitrirattanakul,*[2] [1]*Department of Oral Diagnostic Science, School of Dentistry, University of Phayao* [2]*Department of Masticatory Science, Faculty of Dentistry Mahidol University*
02:15-02:30	Coffee break	
02:30-02:45	OJ-00007	**A Systematic Review of the Effectiveness of Laser Therapy in Prevention of Osteoradionecrosis of the Jaw** ***Thipok SOMBUTSIRINUN,***[1,2,3] *Suwat TANYA,*[2,4] *and Sajee SATTAYUT*[1,2] [1]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand* [2]*Lasers in Dentistry Research Group (LDRG), Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand* [3]*Department of Oral and Maxillofacial Surgery, School of dentistry, University of Phayao, Phayao, Thailand* [4]*Department of Community Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai Thailand*

02:45-03:00	OM-00002	**MRI Evaluation on Articular Disc Position after Mandibular Setback; A Pilot Study** *Nathakarn THAMWATHARSAREE,[1] Wannakamon Panyarak,[2] Kittichai Wantanajittikul,[3] Uten Yarach,[3] and Kathawut Tachasuttirut[4]* *[1]Graduate School, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand* *[2]Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Chiang Mai University,Chiang Mai, Thailand* *[3]Department of Radiologic Technology, Faculty of Associated Medical Science, Chiang Mai University, Chiang Mai, Thailand.* *[4]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand*
03:00-03:15	OM-00003	**Salivary Glucose in Patients with Type 2 Diabetes Mellitus: A Preliminary Study** *Pirayaporn PUANGMALIWAN,[1] Suteera TECHATANAWAT,[2] Bhornsawan THANATHORNWONG,[2] Weerapan KHOVIDHUNKIT,[3] Kongthawat CHAIRATVIT[4] and Siribang-on PIBOONNIYOM KHOVIDHUNKIT[5]* *[1]Master's degree program in Advanced General Dentistry, Faculty of Dentistry, Srinakharinwirot University, Bangkok, Thailand* *[2]Department of General Dentistry, Faculty of Dentistry, Srinakharinwirot University, Bangkok, Thailand* *[3]Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand* *[4]Department of Oral Biology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand* *[5]Department of Advanced General Dentistry, Faculty of Dentistry, Mahidol University, Bangkok, Thailand*
03:15-03:30	OM-00004	**Efficacy of Cannabidiol for Treating Recurrent Aphthous Ulcers** *Chalapinyo UMPREECHA[1] and Kanokporn BHALANG[2]* *[1]Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* *[2]Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand*
03.30-03.45	OM-00007	**Anesthetic Success of Adding Magnesium to Local Anesthetic in Third Molar Surgery** *Yanisa NARUENARTWONGSAKUL,[1] Warit Powcharoen,[2] and Kathawut Tachasuttirut[2]* *[1]Graduate school, Faculty of Dentistry, Chiang Mai University, Thailand* *[2]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chiang Mai University, Thailand*
03.45-04.00	OM-00032	**The Development of Glass Ionomer Cement to Improve Fluoride Release Ability** *Phakvalunch RUJIRAPRASERT,[1] Voravee P. HOVEN,[2,3] Rangsima SAKOOLNAMARKA,[1] and Panida THANYASRISUNG[4]* *[1]Department of Operative Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* *[2]Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand* *[3]Center of Excellence in Materials and Bio-interfaces, Chulalongkorn University, Bangkok, Thailand* *[4]Department of Microbiology and Center of Excellence on Oral Microbiology and Immunology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand*
Conference Room 2 (516) **Oral Presentation 1:00–4:00 P.M. Thailand local time, (GMT+7); Nov 3, 2022** **Chairman: Professor Dr. Chidchanok Leethanakul** **Co-Chairman: Ass. Prof. Dr. Nathawut Kaewsutha**		
01:00-01:15	OM-00008	**The Accuracy of Gold-Reciproc Motor as Electronic Apex Locator during Different Rotation Movements of Rotary Files** *Natthaya SAKSOMBOON,[1] Jaruma SAKDEE,[2] and Suwit WIMONCHIT[2]* *[1]Graduate school, Faculty of Dentistry, Srinakharinwirot University, Bangkok, Thailand* *[2]Department of Conservative Dentistry and Prosthodontics, Faculty of Dentistry, Srinakharinwirot University, Bangkok, Thailand*

01:15-01:30	OM-00019	**Effectiveness of Intra-buccinator 8mg Dexamethasone Injection in Mandibular Third Molar Surgery** *Aung Kyaw OO,[1] Sirichai KIATTAVORNCHAROEN,[1] Kiatanant BOONSIRISETH,[1] Natthamet WONGSIRICHAT,[2] Chakorn VORAKULPIPAT,[1] and Teeranut CHAIYASAMUT[1]* *[1]Department of Oral & Maxillofacial Surgery, Faculty of Dentistry, Mahidol University, Bangkok, Thailand* *[2]Department of Oral & Maxillofacial Surgery, Faculty of Dentistry, Thonburi University, Bangkok, Thailand*
01:30-01:45	OM-00025	**Saliva Profile of Patients with Primary Burning Mouth Syndrome Using ATR-FTIR Spectroscopy** *Florentino Kurt LOZANO III,[1,2] Molin WONGWATTANAKUL,[3] Teekayu Plangkoon JORNS,[1,4] Poramaporn KLANRIT,[1,5] Rajda CHAICHIT,[6] Wilairat SARIDEECHAIGUL[1,4]* *[1]Department of Oral Biomedical Sciences, Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand* *[2]Department of Clinical Dental Health Sciences, College of Dentistry, University of the Philippines, Manila, Philippines* *[3]Division of Clinical Chemistry, Faculty of Medical Technology, Khon Kaen University, Khon Kaen, Thailand* *[4]Neuroscience Research and Development Group, Khon Kaen University, Khon Kaen, Thailand* *[5]Research Group of Chronic Inflammatory Oral Diseases and Systemic Diseases Associated with Oral Health, Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand* *[6]Department of Preventive Dentistry, Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand*
01:45-02:00	OA-00004	**A Novel Alveolar Distractor Incorporating Nickel–Titanium Alloy Springs: A Preliminary *In Vitro* Study** ***Sarun CHANCHAROEN,**[1] Peerapong SANTIWONG,[1] Dutmanee SERIWATANACHAI,[2] Anak KHANTACHAWANA,[3] and Rochaya CHINTAVALAKORN[1]* *[1]Department of Orthodontics, faculty of Dentistry, Mahidol university, Bangkok, Thailand* *[2]Department of Oral biology, faculty of Dentistry, Mahidol university, Bangkok, Thailand* *[3]Department of Mechanical Engineering, faculty of Engineering, King Mongkut's University of technology, Bangkok, Thailand*
02:00-02:15	OA-00012	**The Protective Effect of Stannous Fluoride-Containing Products on Enamel Erosion** ***Siriporn SONGSIRIPRADUBBOON**[1]and Apichaya Jiemkim[1]* *[1]Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand*
02:15-02:30	Coffee break	
02:30-02:45	OA-00014	**The Remineralized Effect on Dentin Caries Using a Light-Cured Silver Diamine Fluoride** *Jutarat PHUENSURIYA,[1] Chutima TRAIRATVORAKUL,[1] Panida THANYASRISUNG,[2] and **Oranuch TECHATHARATIP**[1]* *[1]Department of Pediatric Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* *[2]Department of Microbiology and Center of Excellence on Oral Microbiology and Immunology, Faculty of Dentistry, Chulalongkorn University, Thailand*
02:45-03:00	OM-00001	**Efficacy of Cleaning Solutions on Micro-Shear Bond Strength to Saliva Contaminated Zirconia** *Souphakit NIYOMRATTANAKIT[1] and Teerapong MAMANEE[2]* *[1]Graduate student, Department of Restorative Dentistry and Periodontology, Faculty of Dentistry, Chiangmai University, Chiang Mai, Thailand* *[2]Department of Restorative Dentistry and Periodontology, Faculty of Dentistry, Chiangmai University, Chiang Mai, Thailand*
03:00-03:15	OM-00006	**Effect of Saliva Decontamination Methods on Bond Strength of Developed Zirconia Ceramic** *Tanida KORJAROENRATTANAKUL[1] and Weeranuch THONG-NGARM[2]*

		[1]Graduate student, Department of Restorative Dentistry and Periodontology, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand [2]Department of Restorative Dentistry and Periodontology, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand
03:15-03:30	OM-00014	**Move to Competition**
03.30-03.45	OM-00015	**Microtensile Bond Strength of Resin-Resin Interfaces under Immediate Saliva Decontamination Methods** *Wassamon TORPAIBOON,[1] Watcharaphong ARIYAKRIANGKAI,[2] and Sumana JITTIDECHARAKS[2]* [1]Dentist at Wangnoi hospital, Phranakorn Si Ayutthaya, Thailand [2]Department of Restorative Dentistry and Periodontology, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand
03.45-04.00	OM-00018	**Comparative Study of Fracture Resistance and Failure Mode of a Self-adhesive Composite** ***Juthanan REEKPRAKHON,[1]*** *Kemporn Kitsahawong,[1] Patimaporn Pungchanchaikul[2]* [1]Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand [2]Oral Health Care Center for Geriatric and Special Needs Patients, Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand
Conference Room 3 (515) **Oral Presentation 1:00–3:45 P.M. Thailand local time, (GMT+7); Nov 3, 2022** **Chairman: Professor Vinai Sirichitra** **Co-Chairman: Assoc. Prof. Thongnard Kumchai**		
01:00-01:15	OM-00005	**Urinary and Water Fluoride Levels in Dental Fluorosis Patients in Thailand** *Patcharaporn GAVILA,[1,2] Supoj CHUMNANPRAI,[2] Thantrira PORNTAVEETUS,[1, 3]* [1]Master of Science Program in Geriatric Dentistry and Special Patient Care, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand [2]Intercountry Centre for Oral Health, Department of Health, Ministry of Public Health, Chiangmai, Thailand [3]Center of Excellence in Genomics and Precision Dentistry, Department of Physiology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
01:15-01:30	OM-00027	**Effects of Plant-Derived Epidermal Growth Factor on Salivary Gland Epithelial Cell Proliferation** *Toan PHAN,[1,2] Yamin OO,[1] Teerapat RODBOON,[1] Waranyoo PHOOLCHAROEN,[3] Supansa YODMUANG,[4] and Joao FERREIRA[1]* [1]Avatar Biotechnologies for Oral Health and Healthy Longevity Research Unit, Department of Research Affairs, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand [2]International Graduate Program in Oral Biology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand [3]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand [4]Research Affairs, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
01:30-01:45	OM-00033	**The Immediate Effect of Using Bisdemethoxycurcumin and Potassium Iodide aPDT on *Candida Albicans* Biofilm** *Monique O. RAVAGO,[1,2] Noppawan P. MORALES,[3] Waranuch PHITIPHAT,[4] and Teerasak DAMRONGRUNGRUANG[1,5,6]*

		[1]Division of Oral Diagnosis, Department of Oral Biomedical Sciences, Faculty of Dentistry, Khon Kaen University, Thailand [2]College of Dentistry, University of the Philippines, Manila [3]Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand [4]Division of Dental Public Health, Department of Preventive Dentistry, Khon Kaen University, Khon Kaen, Thailand [5]Melatonin Research Program, Khon Kaen University, Thailand [6]Laser in Dentistry Research Group, Khon Kaen University, Thailand
01:45-02:00	Preparation for Competition Session (Undergraduate)	
02:00-02:15	OC-00007	**Effectiveness of Iodine for Continuous Decontamination of Dental Unit Waterline** ***Pitchayapa SA-NGIAMPAK***,[1] Chatchaya PETCHPHAYAPRAI,[1] Chutimon CHOTIPAN,[1] Kittisak THOTSAPORN,[2] and Ruchanee SALINGCARNBORIBOON AMPORNARAMVETH[1] [1]Research Unit on Oral Microbiology and Immunology, Department of Microbiology,Faculty of Dentistry, Chulalongkorn University [2]Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University
02:15-02:30	OC-00008	**Effects of Multiple Firing on the Color and Translucency of CAD-CAM Glass-Ceramic** ***Jiratchaya Chanuaychai***,[1] Chanakarn Kittipornnont,[1] and Weerakiat Charoensatapon[1] [1]School of Dentistry, Srinakharinwirot University, Bangkok, Thailand
02:30-02:45	OC-00010	**Melatonin Prevents H_2O_2-Induced Premature Senescence in Human Gingival Fibroblasts** ***Krongpong MONPENGPINIJ***,[1] Pnotporn JANTARAKOLICA,[1] Pimlapas THONGANA,[1] Tawepong ARAYAPISIT,[2] and Varunya CHANTADUL[2] [1]Doctor of Dental Surgery student (International program), Faculty of Dentistry, Mahidol University [2]Department of Anatomy, Faculty of Dentistry, Mahidol University
02:45-03:00	OC-00011	**Withdrawn**
03:00-03:15	OC-00014	**Finite Element Analysis of KKU Smart Interlocking Sleep Device for OSA Therapy** ***Kittipit SRISANOI,***[1] Chanissara Thanachaisakul,[1] Pincha Santivivattanaphong,[1] Poonsak Pisek,[2] Yotsakorn Pratumwal,[3] Teekayu Plangkoon Jorns,[4] and Supanigar Ruangsri[5] [1]Dental student, Faculty of Dentistry, Khon Kaen University, Thailand [2]Associated Professor, Department of Preventive Dentistry, Faculty of Dentistry, Khon Kaen University, Thailand [3]Senior engineer, National Metal and Materials Technology Center (MTEC) [4]Associated Professor, Department of Oral Biomedical Science, Faculty of Dentistry, Khon Kaen University, Thailand [5]Assistant Professor, Department of Oral Biomedical Science, Faculty of Dentistry, Khon Kaen University, Thailand
03:15-03:30	OC-00015	**Effect of cavity conditioners on microleakage of zirconia reinforced glass ionomer in class V restoration** ***Yanamon Rujiratorn***,[1] Natjanan Kitkanokudomdach,[1] Ploy Padaeng,[1] Pattraporn Surewong,[1] Sirinart Cheewakriengkrai,[2] and Tadchan Krongbaramee[2] [1]The sixth year dental student, Faculty of dentistry, Chiang Mai University, Chiang Mai [2]Department of Restorative Dentistry and Periodontology, Chiang Mai University, Chiang Mai
03.30-03.45	OC-00016	**Development of Alveolar Ridge Model for the Practice in Mandibular Torectomy** ***Phongsakorn YAOWAPHANKUL***,[1] Phakin CHROENSUBTHAWORN,[1] Nutthamon WONGSOMBAT,[1] Nutnalin SINPISUTH,[1] and Mali NIYOMBANDITH[2] [1]Faculty of Dentistry, Prince of Songkla University, Songkhla, Thailand [2]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Prince of Songkla University, Songkhla, Thailand

Scientific Program of Poster Presentation

November 2-3, 2022

Poster Presentation in DFCT 2022 Interactive Website Exhibition Room (518)	
PA-00001	**Intermittent Compressive Force Regulates DMP1 Expression in Human Periodontal Ligament Stem Cells** ***Jeeranan MANOKAWINCHOKE***[1,2] *Suconta Chareonvit,*[2] *Vorapat Trachoo,*[3] *Phoonsuk Limraksasin,*[1,2] *Hiroshi Egusa,*[4,5] *and Thanaphum Osathanon*[1,2] [1]*Dental Stem Cell Biology Research Unit, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* [2]*Department of Anatomy, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* [3]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand.* [4]*Division of Molecular and Regenerative Prosthodontics, Tohoku University Graduate School of Dentistry, Sendai, Japan* [5]*Center for Advanced Stem Cell and Regenerative Research, Tohoku University Graduate School of Dentistry, Sendai, Miyagi, Japan*
PA-00002	**Formulation of 1% α Mangostin in Orabase Gel Induces Apoptosis in Oral Squamous Cell Carcinoma** ***Wipawee NITTAYANANTA***[1] *Teerapol Srichana,*[2,3] *Jureeporn Chuerduangphui,*[4] *Ekarat Hitakomate,*[1] *and Kesinee Netsomboon*[5] [1]*Faculty of Dentistry, Thammasat University, Pathum Thani, Thailand* [2]*Drug Delivery System Excellence Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand* [3]*Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand* [4]*Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand 5Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Thammasat University, Pathum Thani, Thailand*
PA-00004	**Effect of Adhesive Removing Methods on Shear Bond Strength of Reused Ceramic Brackets** ***Khanumphorn PINGKUL***[1] *Kanokporn Wanitpisitpun,*[1] *Ploynapat Watcharapaha,*[1] *Supakorn Nunnavatanakul*[1] *and Jirawan Chartpitak*[1] [1]*School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand*
PA-00005	**A Study of Suitable Thymol Solubility for use in Dentistry** *Sarut Thairat,*[1] ***Thanattha Wuttihasa,***[2] *Peerapong Tua-Ngam,*[2] *and Supawida Kunlug*[2] [1]*Research Service Section, Research Office, Faculty of Dentistry, Mahidol University, Bangkok, Thailand* [2]*Research Applied Section, Research Office, Faculty of Dentistry, Mahidol University, Bangkok, Thailand*
PA-00006	**Effect of Post-Rinsing Time and Method on Accuracy of Stereolithography Photopolymer Resin** ***Awutsadaporn KATHENG***[1] *Manabu KANAZAWA,*[2] *Yuriko KOMAGAMINE,*[3] *Maiko IWAKI,*[4] *Sahaprom NAMANO,* [3] *and Shunsuke MINAKUCHI*[3] [1]*Department of Restorative Dentistry, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand* [2]*Department of Digital Dentistry, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Yushima, Bunkyo, Tokyo, Japan* [3]*Department of Gerodontology and Oral Rehabilitation, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Yushima, Bunkyo, Tokyo, Japan* [4]*Department of Oral Prosthetic Engineering, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Yushima, Bunkyo, Tokyo, Japan*
PA-00007	**Amelogenesis Imperfecta: Tooth Characteristics in Thai Families** ***Chompak Khamwachirapitak***[1] *Kanokwan Sriwattanapong,*[1] *and Thantrira Porntaveetus*[1] [1]*Center of Excellence in Genomics and Precision Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330, Thailand*

PA-00008	**Factors Influencing the Dietary Behavioral of Preteenagers in Bangkok, Thailand** ***Serena S SAKOOLNAMARKA***[1] [1]*Faculty of Dentistry, Srinakharinwirot University*
PA-00009	**Cytotoxicity of Newly formulated Chlorhexidine mouthwash against Mouse Fibroblasts** ***Peerapong TUA-NGAM***[1] *and Rattiporn Kaypetch*[1] [1]*Research Applied Section, Research Office, Faculty of Dentistry, Mahidol University, Bangkok, Thailand*
PA-00010	**Sex and Age Classification from Radiographic Femur using Machine Learning Algorithms: A Preliminary Study** ***Patara RATTANACHET***,[1] *Wannakamon PANYARAK,*[2] *Kittichai WANTANAJITTIKUL,*[3] *Wattanapong SUTTAPAK,*[4] *and Pasuk MAHAKKANUKRAUH*[5] [1]*PhD student, Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand* [2]*Division of Oral and Maxillofacial Radiology, Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand* [3]*Department of Radiological Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand* [4]*Division of Computer Engineering, School of Information and Communication Technology, University of Phayao, Phayao, Thailand* [5]*Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*
PA-00011	**Antimicrobial action Newly Formulated Chlorhexidine Mouthwash against Oral Pathogens** ***Rattiporn KAYPETCH***[1] *and Peerapong Tua-ngam*[1] [1]*Research Applied Section, Research Office, Faculty of Dentistry, Mahidol University, Bangkok, Thailand*
PA-00012	**Comparative study of Reciprocating NiTi File Systems in Preparing S-shaped Canals** ***Supapit SANGAROON***[1] *and Chinalai PIYACHON*[1] [1]*Department of Conservative Dentistry and Prosthodontics, Faculty of Dentistry, Srinakharinwirot University, Bangkok, Thailand*
PA-00014	**The Trend of Artificial Intelligence Publications in Dentistry** ***Pairin TONPUT***[1] *and Peerapong Tua-ngam*[1] [1]*Research Office, Faculty of Dentistry, Mahidol University, Bangkok, Thailand*
PA-00015	**Effect of Kaempferia parviflora Extract on Proliferation and Migration of Human Gingival Fibroblasts** ***Sarut THAIRAT***,[1] *Varunya CHANTADUL,*[2] *and Supaporn MALA*[1] [1]*Research Office, Faculty of Dentistry, Mahidol University, Bangkok, Thailand* [2]*Department of Anatomy, Faculty of Dentistry, Mahidol University, Bangkok, Thailand*
PA-00016	**Antibacterial Activities of Thai Medicinal Herbal Extracts Against Dental Pathogens** ***Suwanna KORSUWANNAWONG***,[1] *Thaniya MUADCHEINGKA,*[2] *Supaporn MALA,*[1] *and Ratchaporn SRICHAN*[1] [1]*Research Office, Faculty of Dentistry, Mahidol University. Bangkok, Thailand* [2]*Microbiology Department, Faculty of Dentistry, Mahidol University. Bangkok, Thailand*
PA-00018	**Effect of Different Microcrystalline and Paraffin Ratio on the Volumetric Shrinkage of Dental Wax** ***Kunatip SUKPRASERT***,[1] *Natchaya THITIVARAPORN,*[1] *Nichakarn MEKBENJAPIWAT,*[1] *Sukritta RUANGDECH,*[1] *Sitthi DUANGPHET,*[2] *and Komkham PATTANAPORN*[1] [1]*School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand* [2]*School of Science, Mae Fah Luang University, Chiang Rai, Thailand*

PA-00019 Effect of Nicotine on Primary Mouse Embryonic Fibroblasts *in vitro*

Hathairat Lekatana,[1] *Jadesada Palasuk,[2] Suttipalin Suwannakul,[3] Suwimon Jettanacheawchankit,[1] Piyamas Sumrejkanchanakij[4] and Rungarun Kriangkrai[1]*
[1]Department of Oral Biology, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand.
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[3]Department of Preventive Dentistry, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand.
[4]Department of Anatomy, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

PA-00020 Generation Gap Project: An Application Health Promotion Concept for Dental Students

Thikumpond MALISON,[1] *Areerat NIRUNSITTIRAT,[1] and Piyanart CHATIKETU[1]*
[1]Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand

PA-00021 Bond Strength Between Orthodontic Metallic Brackets Bonded to Different Tooth-colored Restorative Material

Dr. Rutapakon Insawak,[1] *Dr. Spun Lenglerdphol,[2] Tamanan Ruangsiri,[3] Thanatham Kengwitkam,[4] Nichakarn Jinaphen,[5] Jinitta Ratimart,[6] and Orarak Choovanichvong[7]*
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PA-00022 Effect of Oxidation Heat Treatment on Oxide Layer Thickness and Bonding Strength of Reused Co-Cr Alloy for PFM

Anucha SACHAROEN,[1] *Chayada TEANCHAI,[1] and Mack BOONPENSIN[2]*
[1]Research Office, Faculty of Dentistry, Mahidol University, Bangkok, Thailand
[2]Tool and Die Technology Center, Faculty of Engineering and Architecture, Rajamangala University of Technology Suvarnabhumi Mueang Nonthaburi District Nonthaburi

PA-00023 Effect of Herbal Extract Toothpaste on Oral Malodor Reduction

Sarut THAIRAT[1] *and Chayada TEANCHAI[1]*
[1]Research Office, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

PA-00024 Wear of Pit and Fissure Sealants after Chewing Simulation

Pimduean SIVAVONG,[1] ***Anucharte SRIJUNBARL,[2]*** *Thantrira PORNTAVEETUS,[3] Thawanrat SINGTHONG,[2] Ekamon MAHAPOKA,[1] and Dusit NANTANAPIBOON[2]*
[1]Department of Operative Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
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PA-00025 Study of Oxidation Behavior on Utilized Co-Cr Alloy Specimens for PFM

Chayada TEANCHAI[1] *and Mack BOONPENSIN[2]*
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PA-00026 Blending Effect of Resin Composites with Surrounding Structures

Leena LEELATIAN[1] and ***Choltacha HARNIRATTISAI***[1]
[1]Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

PA-00027 The Effect of Xylitol in the Presence of Sucrose on *Streptococcus sanguinis*

Panida THANYASRISUNG,[1] *Panadda YUAPIBANRAK,[2] and Oranart MATANGKASOMBUT[1]*
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[2]Medical Microbiology Medical Microbiology, Interdisciplinary Program, Graduate school, Chulalongkorn University, Bangkok, Thailand

PA-00028	**Production and Characterisation of Human Tetrameric Extracellular Superoxide Dismutase** ***Varunya CHANTADUL***,[1] *Gareth S.A. WRIGHT,[2] Svetlana V. ANTONYUK,[3] and S. Samar HASNAIN[3]* *[1]Department of Anatomy, Faculty of Dentistry, Mahidol University, Bangkok, Thailand* *[2]School of Life Sciences, University of Essex, Essex, United Kingdom* *[3]Molecular Biophysics Group, Institute of Systems, Molecular and Integrative Biology, Faculty of Health and Life Sciences, University of Liverpool, Liverpool, United Kingdom*
PA-00029	**Antimicrobial Effects of Bicarbonate on Cystic Fibrosis Bacteria** ***Pongsiri Jaikumpun***,[1] *Kasidid Ruksakiet,[1,2] Balázs Stercz,[3] Éva Pállinger,[4] Martin Steward,[1,5] Zsolt Lohinai,[2] Orsolya Dobay,[3] and Ákos Zsembery[1]* *[1]Department of Oral Biology, Semmelweis University, Budapest, Hungary* *[2]Department of Conservative Dentistry, Semmelweis University, Budapest, Hungary* *[3]Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary* *[4]Institute of Genetics, Cell, and Immunobiology, Semmelweis University, Budapest, Hungary* *[5]School of Medical Sciences, University of Manchester, Manchester, United Kingdom*
PA-00030	**Effect of 1450 ppm Fluoride Toothpaste on Surface Microhardness of Artificial Caries** ***Penarsan SOONTHAROTOKE***[1] *and Nattanan GOVITVATTANA[2]* *[1]Residency Training of Pediatric Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* *[2]Department of Pediatric Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand*
PA-00031	**Effect of Immersing Orthodontic Fixed Appliances into Toothpaste Slurry on Surface Friction** ***Sumit SUAMPHAN***,[1] *Ruangkhaow Pimonsri,[1] Naphaphon Thongmueang,[1] and Krittin Prakairungthong[1]* *[1]School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand*
PA-00032	**Association between Chewing Side Preference and Orientation of Mandibular Teeth in Late Adolescence** ***Sumit SUAMPHAN***,[1] *Salisa Kitsuwannaratana,[1] Thichakorn Chitmaneet,[1] Rutjarat Kanjana,[1] and Piyathida Bumrungrod[1]* *[1]School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand*
PA-00033	**Comparison of Tongue Characteristics Classified According to Ultrasonographic Using K-Means Clustering Algorithm** ***Ariya CHANTARAMANEE***,[1] *Kazuharu Nakagawa,[2] Kanako Yoshimi,[2] Ayako Nakane,[2] Kohei Yamaguchi,[2] and Haruka Tohara[2]* *[1]Department of Preventive Dentistry, Naresuan University, Phitsanulok, Thailand* *[2]Department of Dysphagia Rehabilitation, Tokyo Medical and Dental University, Tokyo, Japan*
PA-00034	**Teaching Empathy Through Interactive Activities for Healthcare Students** ***Yotsawan MUENSAIYAT***,[1] *Piyanart Chatiketu,[1] Pijittra Jankana,[1] Thikumpond Malison,[1] and Areerat Nirunsittirat[1]* *[1]Department of family and community dentistry, Faculty of Dentistry, Chiang Mai University*
PA-00035	**Characteristics and Osteoconductive Property of the Cuttlebone-derived Hydroxyapatite Particles** ***Thanin TANGSUKSANT***,[1] *Sarute Ummartyotin,[2] Thirawudh Pongprayoon,[3] Premjit Arpornmaeklong,[4] and Komsan Apinyauppatham[4]* *[1]Master of Science Program in Dental Implantology, Faculty of Dentistry, Thammasat University, Pathum Thani, Thailand* *[2]Department of Materials and Textile Technology, Faculty of Science and Technology, Thammasat University, Pathum Thani, Thailand* *[3]Department of Chemical Engineering, Faculty of Engineering, King Mongkut's University of Technology, Bangkok, Thailand* *[4]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Thammasat University, Pathum Thani, Thailand*

PA-00036	**Foreign Body Reaction of Dermal Fillers to The Mandible: Case Report** ***Vorapat Trachoo***, *On-a-Nong Silkosetsak and Wichitsak Cholitgul* *Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand*
PA-00037	**Autotransplantation of Premolar to Maxillary Incisor in Orthodontic Patients: A Report of 2 Cases** *Paisan KANGVONKIT,[1] Pisuthorn KANGVONKIT,[2]* ***Nongnaphat LUKSANAWONG****,[1] and Ekapong DECHTHAM[1]* *[1]School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand* *[2]Siripen Memorial Dental Clinic, Chonburi, Thailand*
PA-00038	**Chitosan-Based Scaffold Incorporated with Trichostatin A for Bone Tissue Engineering** ***Teerawat SUKPAITA****,[1] Suwabun CHIRACHANCHAI,[2] Atiphan PIMKHAOKHAM,[3] and Ruchanee Salingcarnboriboon AMPORNARAMVETH[4]* *[1]Department of Oral Surgery, Faculty of Dentistry, Naresuan University, Phitsanulok 65000, Thailand* *[2]Center of Excellence on Petrochemical and Materials Technology, Chulalongkorn University, Bangkok 10330, Thailand* *[3]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330, Thailand* *[4]Department of Microbiology, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330, Thailand*
PA-00039	**A Semi-Rigid Shell Barrier System: An Alternative System for Bone Regeneration** ***Rudjit TUNTHASEN****,[1,2] Prisana Pripatnanont,[2] and Jirut Meesane[3]* *[1]Department of Oral surgery, Faculty of Dentistry, Naresuan University, Phitsanulok 6500 Thailand* *[2]Cranio-Maxillofacial Hard Tissue Engineering Center, Oral and Maxillofacial Surgery Section, Faculty of Dentistry, Prince of Songkla University, Songkhla 90110, Thailand* *[3]Institute of Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Songkhla 90110, Thailand*
PA-00040	**Knowledge, Attitude, and Oral Health Care Practices of Caregivers for Bedridden Patients** *Ariyaporn KAEWDUANGSEANG,[1] Ilada PANICH,[1] Narudee LIMPABANDHU,[1] Wanutchaporn LUCKBOON,[1] Ratchanee MITKITTI,[2] and* ***Komkham PATTANAPORN****[1]* *[1]School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand* *[2]School of Nursing, Mae Fah Luang University, Chiang Rai, Thailand*
PA-00041	**The Dilemmas of Professional Development that Dental Students Face during Clinical Practice** *Tanut Khanpharshay, Thanapat Khamparat, and* ***Sarita Piankij*** *Chiangmai University*
PA-00042	**System Dynamics Model of SSB Tax and Dental Caries in Thai Children** ***Nipaporn URWANNACHOTIMA****[1] and John Pastor Ansah[2]* *[1]Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand* *[2]Health Services and Systems Research, Duke-NUS Graduate Medical School, Singapore*
PA-00043	**High Nitric Oxide Induces Autophagy in Head and Neck Cancer Cells** *Diane Isabel SELVIDO,[1] Sittichai KOONTONGKAEW,[1] and* ***Kusumawadee UTISPAN****[2]* *[1]International College of Dentistry, Walailak University, Bangkok, Thailand* *[2]Faculty of Dentistry, Thammasat University, Pathum Thani, Thailand*
PA-00044	**Comparison Buccal versus Palatal Miniscrews of Maxillary Anterior Intrusion: A FE Study** ***Pavitra WAIKASETKORN****[1], Eduardo Yugo Suzuki[2], Boonsiva Suzuki[3]* *[1]Lecturer, Department of Orthodontics, Faculty of Dentistry, Bangkokthonburi University, Bangkok, Thailand* *[2]Associate professor, Department of Orthodontics, Faculty of Dentistry, Bangkokthonburi University, Bangkok, Thailand,* *[3]Lecturer, Department of Orthodontics, Faculty of Dentistry, Bangkokthonburi University, Bangkok, Thailand*

PA-00045	**Diagnosis of Ankylosis in Permanent Incisors using AnyCheck Device** ***Eduardo Yugo SUZUKI*** *and Boonsiva Suzuki* *Department of Orthodontics, Faculty of Dentistry, Bangkokthonburi University, Bangkok, Thailand*
PA-00046	**Orthodontic Management of Severely Traumatized Maxillary Incisors: 17 years Follow Up** ***Boonsiva SUZUKI*** *and Eduardo Yugo Suzuki* *Department of Orthodontics, Faculty of Dentistry, Bangkokthonburi University, Bangkok, Thailand*
PA-00047	**Smart Springs for Orthodontic Extraction of Impacted Mandibular Third Molars** ***Boonsiva SUZUKI*** *and Eduardo Yugo Suzuki* *Department of Orthodontics, Faculty of Dentistry, Bangkokthonburi University, Bangkok, Thailand*
PA-00048	**Tenting Graft Method for Alveolar Ridge Augmentation using Tooth Autotransplantation: Case Series** ***Eduardo Yugo SUZUKI****, Thongnard Kumchai, Boonsiva Suzuki* *Faculty of Dentistry, Bangkokthonburi University, Bangkok, Thailand*
PA-00049	**The Relationship between Professional and Normative Orthodontic Treatment Needs in Mae Chan Students, Chiang Rai** ***Pratchtakorn SIRIAROONRAT****,[1] Chutimon Wanapantapornkul,[1] Nattanun Khiewkern,[1] Nitikorn Intapong[1], Pisak Ongsirimongkol,[1] and Jirawan Chartpitak[1]* *[1]School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand*
PA-00050	**Comparison of failure modes of two pit and fissure sealants using conventional etch and self-etching primer: An In Vitro study** ***Pimchanok PUTTAKUN****,[1] Anoma RATTANACHAROENTHUM,[2] and Kemporn KITSAHAWONG[2]* *[1]Dental Department, Thong Pha Phum Hospital, Kanchanaburi, Thailand* *[2]Department of Preventive Dentistry, Division of Pediatric Dentistry, Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand*
PA-00051	**Third Molar Presence and Maxillary Posterior Bone Availability in Different Maxillary Types** ***Ritsakul PANJATHAMMAWIT****, Thongnard KUMCHAI, Boonsiva SUZUKI, and Eduardo Yugo SUZUKI* *Department of Orthodontics, Faculty of Dentistry, Bangkokthonburi University, Bangkok, Thailand*
PA-00052	**The Efficacy of Handwashing Gel Containing Banana Peel Extract in Reducing Bacteria on Dentists' Hands** ***Duangkaew KERDSIRI****,[1] Nada Kengpanich,[1] Benyatip Duangsombat,[1] and Pornpimon Kamchai[1]* *[1]School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand*
PA-00053	**The Efficacy of the Coloring Agents from Black Glutinous Rice on Dental Biofilm Staining** ***Ayyamin CHANTASIRIPHAN****,[1] Chanapa Tedcharoen,[1] Nunsinee Nongpiwong,[1] and Suwimon Suwannaat[1]* *[1]School of Dentistry, Mae Fah Luang university, Chiang Rai, Thailand*
PA-00054	**The Efficacy of Mouthwash Containing Essential Oil from Zanthoxylum limonella Alston on Dental Biofilm and Gingivitis Controls** ***Thanyaporn VACHIRAROJPISAN****,[1] Kanokwan Karnchanarungroj,[1] Chinnakrij Posiri,[1] and Phraodara Hongsamsipjet[1]* *[1]School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand*
PA-00055	**Okra Jelly Affecting Self-perceived Xerostomia and Oral Health-related Quality of Life in the Elderly: A Preliminary Study** ***Chaninard WIRIYAPRASITCHAI****,[1] Visarut Thangvaravut,[1] and Witsuta Pongphaladisai[1]* *[1]School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand*

Keynote Speakers

MicroRNA-Based Gene Therapy for Oral and Craniofacial Diseases

Professor Dr. Liu Hong

University of Iowa College of Dentistry, Iowa City, Iowa, USA

MicroRNAs (miRNAs), small non-coding RNAs, serve as post-transcriptional regulators to promote the degradation and/or repress the translation of mRNA through sequence-specific interactions with specific mRNA targets. miRNAs play critical roles in the developmental biology and the pathophysiology of various diseases and have emerged as a promising therapeutic tool for diagnosis and treatment. miR-200 family is a key regulator in epithelial-mesenchymal transition and is actively involved in inflammation and cell differentiation by targeting various transcriptional factors and cytokines. Our research focuses on understanding the roles of miR-200s in craniofacial development, regeneration, and inflammation, aiming to develop miRNA-based gene therapy for oral and craniofacial disorders, including periodontitis, craniosynostosis and calvarial bone reconstruction.

Keystone Pathogen Hypothesis: an interplay of *Porphyromonas gingivalis* with a local Innate Immunity of the Periodontium

Asst. Prof. Dr. Nutthapong Kantrong

Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand

For years, *Porphyromonas gingivalis* has been identified as a member of Red complex inhabiting in the niche surrounded by periodontal tissues. However, recent investigations in the last decade have explored its pathogenicity and this pathogen successfully disrupts the host immunity via the immune subversion of host cell receptors, posing a risk of transitioning from health to disease on the periodontium. In this talk, evidence from both microbiological and host aspects relevant to pathogenesis of periodontal disease will be discussed.

ADAM9: A Promigratory Molecule in Oral Cancer

Professor Dr.Suttichai Krisanaprakornkit
Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand

Oral squamous cell carcinoma (OSCC) is the most common cancer of the oral cavity. It still poses a significant global health problem with high morbidity and mortality rates. In order to treat patients with OSCC more effectively, a “targeted therapy” has been introduced as an adjunctive treatment in addition to conventional surgical and radiation therapies. In this talk, I will present you some of my already published and new findings, regarding *ex vivo* and *in vitro* overexpression of an interesting target molecule, i.e., A Disintegrin and Metalloproteinase 9 (ADAM9), in OSCC tissues and cell lines as well as the associations between ADAM9 overexpression and several clinicopathological characteristics of patients with OSCC. *In vitro*, the functions of ADAM9 in oral cancer cell proliferation, migration, and invasion have been explored, and it was demonstrated that ADAM9 was clearly involved with oral cancer cell migration and invasion. Moreover, involvement of the ADAM9/epidermal growth factor (EGFR)/AKT pathway in oral carcinogenesis has been addressed due to the significance of this pathway in esophageal squamous cell carcinoma. A new line of preliminary evidence from *in vitro* experiments for a possible connection between ADAM9 and the plasminogen activation system, especially the plasminogen activator inhibitor, in oral cancer cells will be discussed as well. Last but not least, an inhibitory effect on oral cancer cell migration *in vitro* using the newly developed monoclonal antibodies against the disintegrin domain of ADAM9 will be demonstrated.

Molecular Biocompatibility - from a Theory to Clinical Applications in Bone and Dental Pulp Tissue Engineering

Professor Dr. Laisheng Lee Chou
Boston University Henry M. Goldman School of Dental Medicine, Boston, Massachusetts, USA

Despite the frequent uses of biomaterials in the clinic and the sophisticated characterizations of material properties, how the chemistry and topography of these materials control the biological response when these materials are implanted in vivo still remains largely unknown. The mechanism underlying the tissue-implant interface reaction is poorly understood because the complete characterization of biological properties of implant materials at cellular and molecular levels are lacking. Current materials have been selected for use because of their relatively inert state, as defined by chemical-mechanical studies and histological examinations of implant specimens. As we move into the new world of modern biology, if we are to bring biomaterials to their full potential, we must bring about a marriage between the fields of Material Science and Cellular and Molecular Biology with the "cutting edge" knowledge of these two very different areas. The intent of this presentation is to present a concept of "molecular biocompatibility" based on the research findings from previous studies to define the cellular response to the biomaterials at molecular levels. With this concept, phenotypic behaviors of the host in response to the biomaterials can be predicted and measured by the activity of major functional genes involved in the tissue-biomaterials interface reactions, such as cell attachment, osteointegration, potential carcinogenesis, and inflammatory responses. Accordingly, a profile of molecular biocompatibility on each class of biomaterials can be established, being served as a biological guideline for modifying the chemical composition and/or surface topography of biomaterials resulting in the material-controlled interface reactions. Examples of translating this new concept into clinical applications in bone and dental pulp tissue engineering will be presented as well.

Regenerative Endodontics: What Does the Future Hold?

Professor Dr. Avina Paranjpe

School of Dentistry, University of Washington, Seattle, Washington, USA

Mesenchymal stem cells (MSCs) have attracted attention over the last few years as a source for tissue engineering, due to their multi-lineage differentiation capability. Dental derived MSCs have been shown to be effective in Regenerative medicine and dentistry. Stem cells of the apical papilla (SCAP) are one of the dental derived MSCs that play an important role in Regenerative Endodontic Procedures (REPs) and are needed for a successful and favorable outcome in REPs. The other components include a scaffold, growth factors and adequate disinfection. However, the success rates of this procedures are variable and unpredictable. There are potentially many factors that could possibly affect the success rates. These factors need to be delineated to better understand this procedure and make it more predictable and effective. These factors include bacteria and their interaction with the immune system during the procedure. It is also important to understand the future of this procedure and its potential uses, not only in dentistry but also in medicine. This presentation will highlight the important aspects of this procedure, the factors and potential components/ players that need to be reconsidered and ongoing and future research trends.

Dental Education in the Post-COVID-19 Era

Asst. Prof. Dr. Supachai Chuenjitwongsa
Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

Abstract

COVID-19 pandemic has accelerated changes in global education. Dentistry and dental education inevitably need to adapt to the new normal in education. Recently, educational change focused on the impact of COVID-19 on personal routines affecting teaching, learning, and assessment. Innovative educational strategies or practice protocols were adopted and implemented to overcome barriers caused by COVID-19. However, other aspects of dental education affected by COVID-19 have not yet received much consideration.

In this session, the impact of COVID-19 on dental education and current adaptations are presented based on three levels: (1) curriculum level – outcome, output, curriculum, input; (2) institutional level – educational support, climate and environment, human resources, infrastructure; (3) external factors affecting dental education – rules and regulations, policies, quality assurance, global trends. Recommendations for future development in dental education are provided.

Bibliography

Supachai Chuenjitwongsa has a background in dentistry, medical education, and dental education. He received Ph.D. in Dental Education from Cardiff University in 2015. Currently, Supachai is Assistant to the President for Academic Affairs, Educational Innovation and Program Quality Assurance at Chulalongkorn University. He teaches several subjects including evidence-based dentistry, biochemistry, professional development. His research interests include competency-based dental education, faculty development, cultural-appropriate education. Supachai also get involved in administrative duties at national and international levels.

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Guest Speakers

The Role of PRMT4 in Murine Posterior Frontal Suture Closure

Dr. Nicha Ungvijanpunya

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Background: Cranial sutures accommodate brain and skull growth during development. *RUNX2* mutation in cleidocranial dysplasia patients has been associated with open metopic suture. Runx2 is a master regulator of bone formation and its activity is regulated by multiple types of post-translational modifications. Protein arginine methyltransferase 4 (PRMT4/CARM1) is the highest expressing PRMT in osteogenic cells. However, its role in osteogenesis is unknown.

Purpose: To investigate the role of PRMT4 in skull formation

Methods: Neural crest cell (NCC)-specific *Prmt4* deletion mice were examined by skeletal preparation, microCT scanning, immunofluorescence staining, RNAScope, Proximity Ligation Assay, DiI-labeled calvaria culture and RNA-sequencing analysis. Protein methylation assays were performed in 293T cells. Migration assay and ChIP-qPCR were conducted using MC3T3-E1 cells.

Results: We identified that PRMT4 methylated Runx2 at four arginine residues and revealed their co-expression and Runx2 methylation in osteoprogenitors at the osteogenic fronts (OF) of posterior frontal (PF) suture. NCC-specific *Prmt4* deletion caused patent PF suture. We further observed delayed osteoprogenitor migration at the OF without changing cell proliferation or apoptosis. In addition, PRMT4 depletion and methylation-deficient Runx2 impaired migration of mouse calvarial preosteoblasts. We observed a significant downregulation of ECM-related genes with enrichment in cell migration genes, and reduced Runx2 enrichment in migration gene promoters by *Prmt4* deletion. We also noted downregulation of *Sp7* and a subset of bone matrix genes, but the number of Runx2+ or Sp7+ cells at the OF did not decline.

Conclusion: PRMT4-methylated Runx2 at the OF is essential for the migration of osteoprogenitors during PF suture closure.

Research and Development of Fluoride Varnish for Geriatric Dentistry

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Thailand started to become an ageing society from 2005, and it becomes an aged society by 2021. Since the 1980s, the Thai government has been planning policies for the care of the expanding older population, and oral health care is one of the primary areas of emphasis. The dental profession has several problems in providing oral care to the elderly, particularly in terms of their general health and physical condition. The adoption of preventive dental protocols for the elderly is required for the maintenance of excellent oral hygiene and to lessen the treatment complexity. One of the seven most frequent dental illnesses in the elderly, root caries is the leading cause of tooth loss. The safety and efficacy of fluoride varnish in preventing dental cavities are well-established, and it has been routinely used for decades. However, all fluoride varnish products used in Thailand are imported, resulting in a large import value; hence, the Dental Innovation Foundation (DIF) has developed the Fluoride Varnish Development Project in 2015. The physical characteristics, toxicity, and biocompatibility of fluoride varnish from Thailand (DIF-FLV) have been created and tested in accordance with the ISO standard for fluoride varnish products. The findings of a multicenter clinical research indicate that the efficacy of DIF-FLV in preventing root caries is equivalent to that of the market standard brand and has no harmful effects on patients. DIF-FLV is now undergoing package design and industrial manufacturing for mass production and will be utilized in mobile dentistry units for the entire nation.

MRGPRX2 Signaling on Mast Cell-mediated Neuroimmune Interaction

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Mast cells (MCs) play a critical role in neuroimmune interactions and tissue inflammation. Recent evidence suggests that the neuropeptide substance P (SP) contributes to neurogenic inflammation and pain through MC activation via a novel G protein-coupled receptor (GPCR), MRGPRX2. However, the mechanisms underlying MRGPRX2 activation by SP has yet to be elucidated. Using pertussis toxin and YM-254890, we demonstrated that SP induces MRGPRX2-mediated calcium mobilization and degranulation via both $G\alpha_i$ and $G\alpha_q$. Furthermore, We found that SP caused β-arrestin recruitment, receptor internalization and desensitization, indicating that SP serves as a balanced agonist for MRGPRX2. Specific residues of MRGPRX2 that are responsible for SP-induced MC activation and regulation were identified using the information obtained from both structural modeling and naturally occurring MRGPRX2 missense variants. MRGPRX2 mutations were generated using site-directed mutagenesis and transiently transfected into RBL and HTLA cells. The effects of these mutations on SP/MRGPRX2-mediated MC responses were determined. Determining the structure and understanding the molecular mechanisms of MRGPRX2 activation might provide novel therapeutic approaches for the management of neurogenic inflammatory diseases and pain.

Oral Health and Nutrition, an Update

Assoc. Prof. Dr. Dunyaporn Trachootham
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Oral health and nutrition are strongly linked throughout the life cycle from pre-natal to elderly periods. Nutrients are important for the development and physiologic function of oral organs. Nutrient deficiency could cause abnormal oral manifestation and may be used as a sign of the diagnosis. Unbalanced diets can contribute to an increased risk of dental and oral diseases such as caries, periodontal diseases, oral cancer, and dental erosion. Furthermore, being underweight, overweight and obese can influence the severity and treatment outcome of dental diseases. The main function of oral organs is to facilitate eating (chewing, swallowing, and sensation for taste and texture of food). Oral diseases, deformity, dysfunction, or oral complication of cancer treatment may affect oral functions and eating ability. Limitation of oral intake capacity affects the type of food and nutrient received, which could lead to malnutrition, poor quality of life, and eventually death. Therefore, oral health care and dental treatment must be combined with appropriate nutrition management. In this talk, I will discuss recent research findings that show the interlink between oral health and nutrition, and innovation to promote both good oral health and nutrition as a whole.

Abstracts for Oral Presentation

OA-00002

ELANE Mutation Altering Dental Pulp Cell Biomolecular Processes

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ABSTRACT

Background: Severe congenital neutropenia (SCN) characterized by abnormally low levels of neutrophils leading to recurrent infections. Variations in the *ELANE* gene encoding neutrophil elastase (NE) are the major cause of SCN. The SCN patients developed oral complications during early childhood including dental infection, chronic ulcers, severe periodontitis, and early tooth loss.

Objectives: The cause of oral complications in SCN patients is unknown whether from only low neutrophil or combination with aberrant dental cells. The aim of this study was therefore to characterize the dental pulp cells with *ELANE* mutation derived from a SCN patient.

Methods: Genetic variant of a SCN patient was identified by exome sequencing. The patient's pulp cells (*ELANE* cells) were determined for gene expression, enzyme activity, proliferation, colony formation, wound healing, apoptosis, ROS, attachment, spreading, and response to lipopolysaccharide.

Results: The patient possessed the heterozygous 12-bp in-frame insertion c. 289_300dupCAGGTGTTCGCC; p.Q97_A100dup in *ELANE*. ELANE cells showed significantly reduced *ELANE* and *SLPI* expression and diminished neutrophil elastase activity. They exhibited impaired ability in proliferation, colony forming, migration, attachment, and spreading with increased ROS formation and cell apoptosis. The *TGFβ1* and *TNFα* levels were significantly increased while *cyclinD2*, *MMP2*, *IL-6*, *IL-8*, and *NF-kB1* were significantly decreased in ELANE cells. Lipopolysaccharide treated ELANE cells were significantly increased *SLPI*, *TNF-α, NF-kB1, IL-6* and *IL-8* expression.

Conclusion: The study reveals new molecular pathomechanism of pulp cells associated with *ELANE* mutation in the SCN patient. We highlight the research gap and potential strategies to explore novel targeted therapy for oral infection.

Keywords: Dental pulp, Inflammation, Neutrophil elastase, Periodontitis, Protease

Oral Presentation

OA-00004

A Novel Alveolar Distractor Incorporating Nickel–Titanium Alloy Springs: A Preliminary In Vitro Study

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ABSTRACT

Objectives: To evaluate the mechanical properties of a new design of alveolar distractor using Nickel-Titanium (NiTi) open-coil springs in producing distraction forces against the tensile forces of porcine attached gingiva to simulate the human gingiva.

Methods: A new alveolar distractor was designed and fabricated with Titanium Grade IV as the tube and plate. A NiTi open-coil springs (Highland and ORMCO) with three level of forces (light, medium and heavy) were cut to 15 mm length. The springs were subjected to the mechanical testing in a 37±1°C water bath by 55% compression with rod holding and 60.67% or 9.1mm compression in distraction tube holding to determine the exertion force from open-coil spring. Ten strips of porcine mandibular attached gingiva were subjected to tensile tests to determine the resistance force. The forces from the springs were compared with the tensile forces from porcine attached gingiva. Data between groups were analyzed with independent sample T-tests (p-value <.05).

Results: This preliminary *in vitro* study introduced a new design of alveolar distractor incorporated with NiTi open-coil springs that could generate light and continuous forces to overcome the resistance from porcine attached gingiva.

Conclusion: The study reveals new molecular pathomechanism of pulp cells associated with *ELANE* mutation in the SCN patient. We highlight the research gap and potential strategies to explore novel targeted therapy for oral infection.

Keywords: Alveolar distraction osteogenesis, Nickel-Titanium, Open coil springs

OA-00005

Cannabidiol Enhances Differentiation and Biomineralization of Primary Human Osteoblasts via the Canonical Pathway

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ABSTRACT

Background and Objectives: Cannabidiol (CBD), a non-psychological compound of Cannabis sativa, is widely used in medicine due to its various biological properties. Periodontitis is a common oral disease that causes alveolar bone destruction and tooth loss. The aim of this study was to investigate the osteogenic-inducing effect of CBD in primary osteoblasts harvested from human alveolar bone.

Methods: Upon treatment with various doses of CBD, cell viability and proliferation were first determined by the MTT and BrdU assays, respectively. The non-toxic doses of CBD were chosen to treat the osteoblasts in an osteogenic condition. The alkaline phosphatase (ALP) assay, Alizarin red staining, and von Kossa staining were performed to determine osteoblast differentiation and biomineralization. mRNA and protein expressions of several osteoblast-specific genes were analyzed by real-time PCR, immunoblotting, and immunofluorescence.

Results: Treatment with CBD up to 10 mM did not cause any cytotoxic effect, whereas treatment with CBD at 0.01-10 mM was found to significantly enhance osteoblastic proliferation (p<0.05). Treatment with CBD at 1, 3, or 10 mM under an osteogenic condition further increased osteoblastic differentiation and biomineralization significantly (p<0.05); consistently, treatment with CBD significantly enhanced the mRNA expressions of RUNX2, bone sialoprotein, osteocalcin, ALP, and osterix (p<0.05). Furthermore, enhanced expression and accumulation of b-catenin, a downstream molecule of the canonical osteogenic pathway, were found within the osteoblasts with significant increases in its gene and protein expressions (p<0.05).

Conclusion: All findings suggested that CBD can induce osteogenesis in primary osteoblasts obtained from human jaws, possibly via the b-catenin-dependent canonical pathway.

Keywords: Alveolar bone, b-catenin, Cannabidiol, Osteoblasts, Periodontitis

Oral Presentation

OA-00007

The Study of PD-L1 and RPS6 Expression in Oral Epithelial Dysplasia and Squamous Cell Carcinoma

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ABSTRACT

Objectives: Ribosomal protein S6 (RPS6), a downstream component of the mTOR pathway, is upregulated in many cancers including oral squamous cell carcinoma (OSCC). Moreover, programmed death ligand-1 (PD-L1) suppresses anti-tumor immunity and involves in the tumor escape. However, the role of RPS6 and PD-L1 in the progression of oral epithelial dysplasia (OED) to OSCC remains controversial, and the association between RPS6 and PD-L1 is unclear. The aim of the study was to investigate the expression levels of RPS6 and PD-L1 in OSCC and OED, and examine its relationship with different clinicopathological features.

Methods: Fifty-two OSCC and 48 OED cases were recruited for phosphorylated RPS6 (p-RPS6) and PD-L1 immunohistochemical studies. The percentage positivity and intensity of RPS6 and PD-L1 expression were examined and compared between OSCC and OED samples. The correlation between the expression of markers and clinicopathological features of OSCC and OED patients was analyzed.

Results: We found p-RPS6 expression in all cases of OSCC and OED, whereas PD-L1 was expressed in 42/48 (87%) OED and in 28/52 (53%) OSCC. The patients with mild OED presented higher expression level of PD-L1 and p-RPS6 significantly, when compared to moderately-differentiated OSCC patients ($P < 0.002$). The expression level of both p-RPS6 and PD-L1 was not correlated with any clinical parameters.

Conclusion: Our findings suggest that PD-L1 can be expressed in OED and could activate the tumor progression via mTOR pathway.

Keywords: Programed cell death ligan-1, Ribosomal protein S6, Oral epithelial dysplasia, Oral squamous cell carcinoma

OA-00008

Reproducibility of Automated Deep Learning Based Localization of Mandibular Canal on CBCT

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ABSTRACT

Background and Objectives: Reproducibility is key issue in radiological Deep Learning research. The aim of this study is to prove reproducibility and clinical suitability of a previously introduced deep learning system [DLS] for mandibular canal localization in multiple CBCT scans of the same patients. Accuracy, robustness and generalizability of the DLS has been proved previously.

Methods: DLS performance was tested using 83 CBCT scans of 35 patients, 166 mandibular canals. The patients were divided in two groups according to the presence of TMJ prostheses. The mandibular canals were annotated by a senior radiologist as a gold standard, The DLS model outcome was compared to the radiologist's annotations qualitatively and quantitatively. Three other radiologists evaluated qualitatively twice the human and DLS performance giving a 5-point Likert score to assess the suitability for clinical use. Inter- and intraobserver agreement was calculated using Kendall rank correlation coefficient test. For quantitative analysis Mean Curve Distance [MCD], Dice Similarity coefficient [DSC] were reported.

Results: For the Likert score, 98.1% of human annotations and 94.2% of model outputs were clinically found to be suitable. The intraobserver agreement ranges moderate 0.65- 0.58 and the interobserver agreement ranges good 0.95 - 0.77. The median MCD was 0.68 mm. and the median DSC was 0.58 having no significant differences between patient groups.

Conclusion: The DLS gives good results in reproducibility and clinical suitability, and can be used for clinical trials.

Keywords: Automated segmentation, Cone Beam Computed Tomography, Deep learning, Mandibular canal, Reproducibility

Oral Presentation

OA-00010

The Efficacy of Drinking Green Tea on Bone Healing Following Surgical Removal of Impacted Lower Third Molars: A Radiographic Evaluation

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ABSTRACT

Background: Tea contains high levels of Epigallocatechin gallate (EGCG), which has ability to promote bone healing. However, no clinical study shows the efficacy of drinking green tea on bone healing following surgical removal of impacted lower third molars. The aim of this study is to evaluate the effect of drinking green tea on bone healing after surgical removal of impacted lower third molars.

Methods: Orthopantomograms were taken from 18, including patients pre-operative and post-operative (4-8 weeks) of surgical removal of mandibular third molars under similar conditions. The bone healing process was evaluated by the relative bone density, radiographic bone height (RBH), and radiographic infrabony defect (RID). Paired t-test and Independent t-test were used to analyze.

Results: The RBH and the RID showed no statistically significant difference between original and short follow-up in both drinking green tea and non-drinking green tea groups. In addition, relative bone density at a short follow-up postoperative period showed a higher value in the green tea group than the measurement of the non-green tea group. Nevertheless, there is no significant difference.

Conclusion: Drinking green tea investigated in this study positively affects bone healing following surgical removal of impacted lower third molars.

Keywords: Green tea, Third molar, Surgical removal, Bone healing, Orthopantomograph

OA-00012

The Protective Effect of Stannous Fluoride-Containing Products on Enamel Erosion

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ABSTRACT

Objective: To compare the protective effect of three commercial stannous-containing mouth rinses on enamel erosion in a simulated 5-day in vitro cycling model.

Methods: Ninety human enamel specimens were divided into nine groups, group 1: 1450SnF_2 toothpaste alone, group 2, 3, and 4 were 1450SnF_2 toothpaste plus Elmex®, PerioMed™, and Meridol® mouth rinse, respectively, group 5: 1000SnF_2 toothpaste alone, group 6, 7, and 8: were 1000SnF_2 toothpaste plus Elmex®, PerioMed™, and Meridol® mouth rinse, respectively, group 9: the negative control. The erosive challenge was performed with 1 min hydrochloric acid 3 times per day. Toothpaste slurry twice a day for two minutes each, plus a mouth rinse once a day for one minute was applied. Enamel loss was determined by Knoop surface hardness and non-contact profilometry. Finally, enamel surfaces (n=2/group) were examined with a scanning electron microscope.

Results: All groups demonstrated increasing surface loss throughout the experiment. The combination use of toothpaste, either 1000SnF_2 or 1450SnF_2, with stannous mouth rinse provided better erosion protection when compared to toothpaste alone. In both toothpaste concentrations, there was no statistically significant difference in enamel loss among all three mouth rinses groups. However, after five days, only Elmex® and PerioMed™ exhibited significantly less enamel loss than toothpaste alone.

Conclusion: Under this in vitro study, there was no significant difference in reducing enamel loss among all three mouth rinses groups. The combination of 1450SnF_2 toothpaste and Elmex® or PerioMed™ mouth rinse demonstrated a remarkable protective effect against enamel erosion.

Keywords: Enamel, Erosion, Mouth rinse, Stannous fluoride, Toothpaste

Oral Presentation

OA-00014

The Remineralized Effect on Dentin Caries Using a Light-Cured Silver Diamine Fluoride

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ABSTRACT

Background and Objectives: Silver diamine fluoride (SDF) is a material for arresting dentinal caries. The objective of this study was to compare the percentage of mean mineral density change (%MD change) on artificial dentin caries treated with different time SDF applications and light curing on SDF.

Methods: Tooth sample obtained from 10 non-carious permanent molar teeth. Each tooth was cut into 4 dentin slices. The dentin slices were created as artificial dentin caries lesions and randomly assigned into 4 groups (n=10/group): 1) SDF60, applied SDF for 60 seconds (s), 2) SDF10, applied SDF for 10s, 3) SDF60+LC, applied SDF for 60s with light-curing for 20s and 4) SDF10+LC, applied SDF for 10 s with light-curing for 20s. The samples underwent bacterial pH-cycling for 7 days. Baseline and post-pH cycling specimens were scanned using micro-computed tomography (micro-CT) for analysis of the %MD change. The %MD change of the dentin lesions was analyzed using two-way repeated measures ANOVA, least significant difference.

Results: Both the light-curing and the application time factors influenced the %MD change, but these two factors did not interact (p=0.576). The light-curing SDF (SDF60+LC and SDF10+LC groups) resulted in a significant higher %MD change than the no light-curing SDF (p=0.026). The SDF60 group had a significant difference in %MD change with SDF10 group (p=0.020) but did not differ from SDF10LC group (p=0.369).

Conclusion: After light-curing on 10s SDF application, the application time was reduced. The efficiency of remineralization was no different from 60s SDF application.

Keywords: Dentin caries, Light-curing, Remineralization, Silver diamine fluoride

OA-00016

The Development of New Appropriate Horizontal X-Ray Beam Angulation Image Receptor-Holding Instruments

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ABSTRACT

Background and Objectives: This study aimed to develop new appropriate horizontal X-ray beam angulation image receptor-holding instruments, based on the anatomical data of posterior region interproximal surfaces from archived CT images.

Methods: CT images of 92 patients with sound upper and lower dental arches were collected from our CT database and analyzed to determine the angles between the tangential interproximal contact line and the central groove line of posterior teeth. The average angle for each site was calculated and used to modify instruments using a three-dimensional printer. The utilities of the conventional and the modified instruments for viewing proximal surfaces were compared using two dry skulls.

Results: The right and left sides of each site, except for the lower second premolar and first molar sites, did not differ significantly. The difference between the sites was 2.0°; hence, we calculated mean values for the two sides at each site. In the maxilla, the angles of the first and second premolar, second premolar and first molar, and first and second molar to the groove line were 83.9° (±5.4°), 84.4° (±3.9°), 81.6° (±5.1°), while those in the mandible were 85.0° (±9.2°), 85.0° (±4.0°), and 90.6° (±4.9°), respectively. The holding instruments modified to 80° demonstrated better proximal viewing ability in the upper molar region than conventional instruments.

Conclusion: The image receptor holding-instruments were modified according to these angles to allow appropriate X-ray angulation, which facilitated improved observation of the proximal surfaces of teeth in the posterior region in this pilot in vitro study.

Keywords: Dental radiography, Holding instruments, Periapical radiograph, X-ray beam

Oral Presentation

OA-00017

Empathy Level Towards Patients Among Thai Dental Students

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ABSTRACT

Background and Objectives: To develop the Jefferson scale of Empathy-Health Professions student version (JSE-HPS) in the Thai version and assess the empathy level in dental students across gender, universities, and year of dental education.

Methods: A cross-sectional study was conducted among 439 dental students from five public universities and one private dental school in Thailand. JSE-HPS was translated in order to develop the draft Thai JSE-HPS version and was administered to 5 dental students for a pilot test. The final questionnaires (JSE-HPS) included 20 items with a seven-point Likert scale and were interviewed by undergraduate dental students. The internal consistency and test-retest reliability of the questionnaires were tested by using Cronbach's alpha and intraclass correlation coefficient (ICC). Factor analysis was used to examine the underlying factors of the JSE-HPS (Thai language).

Results: The JSE-HPS represented good internal consistency (Cronbach's α = 0.83). Factor analysis revealed, "Compassionate Care", "Perspective Taking" and "Ability to stand in Patients' Shoes" as first, second, and third factors, respectively. The mean empathy score of dental students was 114.30 (SD=13.06) from the total score of 140. There were no significant differences in other factors affecting the empathy levels under different genders, study programs, phases of training, grades, universities, regions, types of university, and years of study.

Conclusion: The mean JSE-HPS score among Thai dental students is higher than those reported in other dental studies. Integrating empathic elements into the dental curriculum will help student learning to be more effective and improve the treatment outcomes.

Keywords: Empathy, Dental students, Jefferson Scale of Empathy, Reliability

OA-00018

Morphological Analysis of Cementoenamel Junction Types in Premolars

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ABSTRACT

Background and Objectives: Cementoenamel junction is a boundary representing the transfer of the inner dentin protective material between the enamel overlapping the crown and the cementum overlapping the root - an important reference point in clinical dentistry. This study aims to observe the correlation between the premolars' enamel, dentin, and cementum at the cementoenamel junction.

Methods: The cervical region of ground sections of 40 premolars that had been extracted for orthodontic reasons were analyzed using transmitted light microscopy to identify which of the following tissue interrelationships was present at the cementoenamel junction: cementum overlapping enamel; enamel overlapping cementum; the edge-to-edge relationship between cementum and enamel; or the presence of gaps between the enamel and cementum with exposed dentin. Research variables include correlation between cementum and dentin and types of correlations between the lingual and buccal surface.

Results: Cementum overlapping enamel was the most common type (39,13%), followed by an end-to-end approximating cementoenamel junction (36,23%), then which the absence of contact between enamel and cementum and revealing a strip of exposed dentin (17,39%) and the rarest type is cementum overlapped by enamel (7,25%). There were no significant differences in correlations between the lingual and buccal surface, and between the maxillary and mandibular ($p>0.05$).

Conclusion: The distribution of mineralized tissues at the cementoenamel junction was varied and unpredictable. Cementum overlapping enamel correlation is the most popular type. It revealed no statistically significant differences in correlations between lingual and buccal surface, maxillary and mandibular.

Keywords: Cementoenamel junction, Ground section teeth, Morphology

Oral Presentation

OA-00019

Extracellular pH Modulates the Second Messenger Concentrations in *Streptococcus mutans*

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ABSTRACT

Background and Objectives: Second messengers (SCs) including cyclic-di-GMP and cyclic-di-AMP determine biofilm formation. *Streptococcus mutans*, a cariogenic bacterium, can survive under extreme acid conditions due to its acid tolerance mechanisms by forming the biofilm. Therefore, we aimed to investigate the effects of different pH values on intracellular SC levels related to the biofilm formation of *S. mutans*.

Methods: *S. mutans* ATCC 35668 was grown in Brain Heart Infusion (BHI) medium pH 7.5 until stationary phase, then exposed to new BHI pH 4.5, 5.5, 7.5, or 8.0 for 0.5 and 3 hours. The SCs were extracted by heat and ethanol, whereas cell pellets were investigated for total cellular protein by Quant-iT® assay. SCs were identified and quantified by HPLC-MS. They were normalized by total protein concentrations. The biofilm formation was measured by crystal violet assay at 48 h. The means ± SD of SCs and biofilm values are analyzed using ANOVA and Tukey's multiple comparisons test ($P < 0.05$).

Results: BHI pH 8.0 significantly reduced c-di-GMP compared to pH 7.5. However, acidic pH did not change c-di-GMP levels. Both acidic and basic pH decreased c-di-AMP levels compared to pH 7.5. Additionally, biofilm formation decreased from alkaline to acid.

Conclusion: Biofilm formation decreased with decreasing pH related to reduced c-di-AMP. Alkaline pH reduced all SCs but increased biofilm formation. The extracellular pH affects all tested second messengers.

Keywords: Biofilm, *Streptococcus mutans*, Acid tolerance, Dental caries, Second messenger

OA-00020

Substrate Stiffness Regulates Proinflammatory Responses in Gingival Fibroblasts

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ABSTRACT

Background and Objective: Thin gingival phenotype or soft gingiva often experiences gingival recession and inflammation; however, the underlying biological mechanisms remain unknown. Extracellular matrix (ECM) stiffness regulates cellular mechanotransduction, which is involved in the progression of fibroblast-related inflammatory disorders in several tissues. We hypothesized that soft gingiva facilitated proinflammatory responses in gingival fibroblasts via cellular mechanotransduction. This study aims to investigate the effects of substrate stiffness on proinflammatory responses and cellular mechanotransduction in gingival fibroblasts (GFs).

Methods: Human GFs (hGFs) from healthy individuals were cultured with or without lipopolysaccharide (LPS) to induce inflammation on type I collagen-coated polydimethylsiloxane substrates with different stiffnesses, representing soft (5 kPa) or hard gingiva (26 kPa). Expression levels of proinflammatory mediators, gingival ECM markers, focal adhesions and mechanotransduction- and inflammation-related transcriptional factors were determined.

Results: Expression levels of proinflammatory mediators such as prostaglandin E2 and interleukin 1 beta in hGFs at 12 h after seeding were significantly higher with the soft substrate than with the hard substrate regardless of LPS stimulation. The soft substrate reduced the expressions of gingival ECM and collagen cross-linking-related markers in hGFs on days 7 and 14. The soft substrate suppressed the focal adhesion assembly and mechanotransduction-related transcriptional factors, but activated the expression of inflammation-related factors. In contrast, the hard substrate induced the opposite reactions in the hGFs.

Conclusion: Soft substrate induced proinflammatory responses and inhibited ECM synthesis in hGFs by inactivating cellular mechanotransduction that highlights the clinical significance of the gingival phenotype as a risk indicator for gingival degradation.

Keywords: Extracellular matrix, Cytokines, Inflammation, Mechanotransduction, Mucosal immunity

Oral Presentation

OA-00021

RANKL-Polarized Macrophages in Bone Healing: *In Vitro* and *In Vivo* Studies

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ABSTRACT

Background and Objective: Receptor activator of nuclear factor kappa-B ligand (RANKL) plays important roles in immune system and bone healing. RANKL induces osteoclast differentiation, however, its effects on macrophage polarization have remained inconclusive. Moreover, denosumab (anti-RANKL antibody) prescribed for osteoporosis and metastasis treatments leads to an accumulation of necrotic bones due to the RANKL inhibition in medicationrelated osteonecrosis of the jaw (MRONJ). Nevertheless, the effects of anti-RANKL antibody on macrophage polarization in MRONJ have remained elusive. The objective of this study is to investigate the roles of the presence and the absence of RANKL in macrophage polarization.

Methods: To determine the effects of anti-RANKL antibody, immunofluorescence staining was conducted in the tissues of the MRONJ mouse model and the M1/M2 ratio was calculated. To study the effects of RANKL on macrophage polarization *in vitro*, murine bone marrow derived macrophages (BMDMs) were treated with RANKL for 2, 6, and 24 hours. RNA-sequencing and RT-qPCR were used to identify the effects of RANKL on M1 and M2-related genes and novel genes.

Results: M1/M2 ratio was significantly increased in the *in vivo* MRONJ model. Interestingly, RANKL had the small but statistically significant upregulatory effect on M1-related genes, however, in the much lower level compared the effects of classical M1 inducers *in vitro*.

Conclusion: Although only the slight effect of RANKL on macrophage polarization was observed *in vitro*, the M1/M2 ratio was significantly increased *in vivo* suggesting the indirect effects of RANKL and/or other mechanisms on macrophage polarization which may potentially contribute to MRONJ pathophysiology.

Keywords: MRONJ, Osteonecrosis, Tooth extraction, Denosumab, Antiresorptive drug

OA-00022

Discrepancies in Parent-Child Reports on Children's Oral Health-Related Quality of Life

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ABSTRACT

Background and Objective: Most questionnaires assessing oral health-related quality of life (OHRQoL) in preschool children are based on proxies which may underestimate children's problems. The Scale of Oral Health Outcomes for Five-Year-Old Children (SOHO-5) is the recently developed questionnaire evaluating children's OHRQoL from children's and parents' viewpoints. In Thai population, the disparities between both viewpoints have not been studied. Therefore, the purpose of this study was to compare parent-child responses on the OHRQoL.

Methods: This cross-sectional study recruited 5-year-old children and their parents from 10 primary schools in Bangkok. The Thai version of SOHO-5 (Th-SOHO-5) was used to measure the impacts of children's oral health problems on eating, drinking, playing, speaking, smiling, and sleeping. Children were interviewed and their parents self-administered the questionnaire. Intraclass Correlation Coefficients (ICCs) analyzed parent-child agreement.

Results: A total of 251 child-parent pairs with mean dmft 4.93 (S.D. = 4.34) participated in this study. The number of children who reported that oral health problems influenced their OHRQoL was greater than that reported by their parents. The average ICC for parent-child reports was 0.47 (95% CI, 0.32-0.59), indicating moderate agreement. Difficult eating was the most troublesome activity from both perspectives (ICC = 0.58, 95% CI; 0.44–0.67). Avoiding smiling due to appearance was another distress for children, but fewer parents perceived it (ICC = 0.25, 95% CI; 0.39-0.41).

Conclusion: There was a discrepancy between parent-child dyads reporting the child's OHRQoL. Assessing the OHRQoL of five-year-old children may require both perspectives in order to thoroughly comprehend the children's problems.

Keywords: Oral health, Quality of life, Dental caries, Child, Discrepancy

Oral Presentation

OJ-00001

Protein Expression after Gingival Injection of mRNA Encoding Platelet-derived Growth Factors-BB in Ligature-induced Periodontitis Model in Rats

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ABSTRACT

Background and Objective: As a new class of medicines, mRNA platform has proven to be safe and effective. Our research group is working on mRNA encoding growth factors for periodontal regeneration. The objective of this study was to investigate protein expression after local administration of mRNA encoding platelet-derived growth factor-BB encapsulated in lipid nanoparticles (PDGF mRNA) in ligature-induced rat periodontitis.

Methods: 3-0 silk was placed around maxillary left second molar for two weeks and then removed, while maxillary right second molar was left non-ligated. A significant bone loss analyzed by stereomicroscope and micro-computerized tomography and gingival bleeding at the ligature sites were observed as compared to the non-ligature sites. To evaluate transfection and protein translation, different doses of PDGF mRNA including low dose (3 µg), medium dose (10 µg), high dose (30 µg), and DPBS (control) and LNPs alone were injected into rat palatal gingiva. The translated PDGF protein production was assessed 24 hours after injection using enzyme-linked immunosorbent assay (ELISA).

Results: High levels of PDGF production were detected at both ligature and non-ligature sites in mRNA treated group. PDGF protein expression showed a trend of dose response but the differences between doses were not significant. Clinical findings at injected sites showed no erythema or swelling. The histological findings showed no evidence of LNPs and other foreign substances of mRNA formulation remaining in the tissues.

Conclusion: This study of 24 hour-local delivery of PDGF mRNA-LNPs into rat periodontitis results in high translated PDGF protein without profound local inflammatory response.

Keywords: Ligature-induced periodontitis, LNPs, mRNA, PDGF, Periodontitis

OJ-00002

Sensitivity of Brux Checker® in Grinding Bruxer

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ABSTRACT

Untreated bruxism can cause pathologic consequences to the components of the masticatory system leading to unnecessary treatments that are often complicated and costly. However, the damage could be prevented if the condition is diagnosed early. Bruxism patients with asymptomatic or mild jaw symptoms usually refused to admit that they grind their teeth. Therefore, a tool with high sensitivity, reasonable price and is comfortable to wear would be beneficial for early screen or diagnosing sleep bruxism. The aim of this clinical study is to investigate Bruxcore Plate (Brux Checker®)'s accuracy to diagnose sleep bruxism in known cases. Forty-four sleep bruxism subjects with clear evidence of bruxofacets on the intraoral appliance were enrolled. Results showed that Brux Checker® had a sensitivity of 84.1 % after one night of application and a consecutive sensitivity of 100 % on the fourth night. In conclusion, at least four nights of Brux cheker® wearing is recommended for sleep bruxism diagnosis.

Keywords: Intraoral device, Oral parafunction, Diagnostic sensitivity, Grinding bruxer, Sleep bruxism

Oral Presentation

OJ-00003

Expression of CLLD7 and CHC1L Proteins in Oral Potentially Malignant Disorders in a Group of Thais: A Preliminary Study

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ABSTRACT

Background and Objective: Chronic lymphocytic leukemia deletion 7 (CLLD7) and chromosome condensation 1-like (CHC1L) proteins are putative tumor suppressor proteins that have never been studied in oral potentially malignant disorders (OPMDs). This study aimed to evaluate the expression of these proteins in OPMDs which were diagnosed according to histopathological diagnosis as acanthosis, mild, moderate and severe epithelial dysplasia, and oral lichen planus/oral lichenoid lesions (OLP/OLL) using immunohistochemistry.

Methods: Six tissue specimens from each group of normal oral mucosa (NOM), acanthosis, mild, moderate and severe epithelial dysplasia, and OLP/OLL were subjected to immunohistochemistry for CLLD7 and CHC1L. Random areas were selected, photographed then at least 1000 cells were counted. Positive cells at different locations were evaluated among groups with statistical significance at $p<0.05$.

Results: CLLD7 and CHC1L proteins were expressed in all groups. For CLLD7, nuclear staining was significantly lower in the severe epithelial dysplasia and the OLP/OLL groups compared to the acanthosis group. The mild and moderate epithelial dysplasia groups exhibited statistically higher number of cells with cytoplasmic staining compared to the NOM group. For CHC1L, the percentage of nuclear staining was reduced, whereas membrane staining was increased in all OPMD groups compared to the NOM group. However, statistically significant difference was observed between the severe epithelial dysplasia and OLP/OLL groups compared to the NOM group.

Conclusion: The altered subcellular localization of CLLD7 and CHC1L in OPMDs suggests that the expression of these tumor suppressor proteins might be dysregulated during malignant transformation processes of oral cancer.

Keywords: CLLD7, CHC1L, Normal oral mucosa, Oral epithelial dysplasia

OJ-00004

The Efficiency of Reducing the Dispersion Aerosols by using Various Types of Intraoral and Extraoral Suction

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ABSTRACT

Background and Objective: COVID-19 is an emerging disease that puts aerosolized dental treatments at a high risk of transmission; up-to-date knowledge of aerosol control plays an essential role in determining standard regulation in dental practice. The objective of this study was to compare the efficiency of reducing the dispersion of aerosols by using various types of suctions.

Methods: A high-speed handpiece was used to create an aerosol. The various types of intraoral and extraoral suctions were divided into six groups. The relative humidity was monitored at 1 min, 5 min, and 10 min with a hygrometer and was triplicated.

Results: Using the saliva ejector alone had the most aerosol diffusion outside the mouth observation. The mean of relative humidity was highest and was statistically significantly higher than other groups that used of a saliva ejector in combination with other suctions. After 1 minute of the procedure, the mean relative humidity in the group using the saliva ejector plus extraoral suction had statistically significantly lower than the group using the saliva ejector plus the high-power suction tip. When the saliva ejector was used with the extraoral suction, the mean relative humidity was no difference between groups after 5-10 mins.

Conclusion: This study provided information for considering investments in the purchase of worthwhile instruments as needed and the most effective in reducing the dispersion of aerosols. The knowledge from this research could be used as a guideline to improve the workflow or regulation in dental practice for safety.

Keywords: Dental aerosol, COVID-19, Dental aerosol reduction, Aerosol control

Oral Presentation

OJ-00006

CREATING A GINGIVAL COLOR DATABASE AMONG THAI SAMPLES BY USING DIGITAL IMAGES PROCESSING

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ABSTRACT

This research aims to 1) analyze the digital color-coded values of clinical gingival color photographs and 2) create a gingival color database using digital photos to measure the color levels of clinical gingival. The research model was cross-sectional descriptive. There were two research methods: 1) digital gingival color code validity analysis and 2) digital gingival color reliability analysis. In the first part, the 99 patients were taken photographs of the gingiva. Adobe Photoshop CS6 software was employed for digital color code analysis. Then, the digital color code of the gingival was examined the reliability by the 143 samples. The present study showed that the total numbers of digital areas from the gingival photos were 3,700 areas, with slightly red being the majority, 35.51 percent, followed by pale pink, red, and bluish-red, for 27.46, 25.43, and 11.59 percent, respectively. The mode of gingival color-coding in both the RGB and HSB color models had 1 or 2 codes, except for the red code that the RGB model had ten and more codes in the HSB model. The reliability analysis of digital gingival was calculated with the alpha coefficient of 0.655, which is a moderate confidence level. Analysis of dental images in the present study has the potential to create a database of gingival color. The digital photo databases are created on the Windows operating system and Microsoft Access software. Performing the software, users can input data consisting of gingival photos, color codes, filter inputs, and search the database of RGB and HSB color models.

Keywords: Gingival color, Digital image analysis, Gingivitis

OJ-00007

A Systematic Review of the Effectiveness of Laser Therapy in Prevention of Osteoradionecrosis of the Jaw

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ABSTRACT

Background and Objective: Osteoradionecrosis of the jaw is one of the complications after the patients received radiotherapy at the head and neck regions. The success rate of current treatments has been unpredictable. Therefore, the prevention of osteoradionecrosis of the jaw is worth considering. The purpose of this systematic review was to evaluate the effectiveness of laser therapy in prevention of osteoradionecrosis of the jaw.

Methods: A systematic review was conducted in published articles in databases of MEDLINE, Embase, Cochrane Library, Scopus, Google scholar, and Thai- Journal Citation Index Center to identify the eligible studies stating the effectiveness to prevent osteoradionecrosis of the jaw. The included studies were assessed with two independent reviewers by using the Cochrane Risk of Bias Tool for randomized controlled trials or the Joanna Briggs Institute critical appraisal for case reports. The agreement of assessment was 90%.

Results: There were 24 articles included by title and abstract. 5 articles were discarded because of duplication. From 19 articles, there were 4 studies met the eligible criteria. It was composed of 1 randomized controlled trial and 3 case reports. From the assessment of the bias, there were low risk.

Conclusion: In conclusion, there was a possibility of using laser therapy immediately after extraction to prevent osteoradionecrosis of the jaw by gaining faster tissue coverage. The combinations of using laser therapies; photobiomodulation and photodynamic therapy with antibiotics or pentoxifylline and tocopherol or PRF, allowed favorable clinical outcomes in prevention of osteoradionecrosis of the jaw.

Keywords: Osteoradionecrosis, Jaw, Laser therapy, Photobiomodulation, Photodynamic therapy

Abstracts for Poster Presentation

10th Anniversary
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PA-00001

Intermittent Compressive Force Regulates DMP1 Expression in Human Periodontal Ligament Stem Cells

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Poster Presentation

ABSTRACT

Background and Objective: Mechanical force differentially regulates periodontal ligament functions depending on types, magnitudes, and duration of stimulation. Intermittent compressive force (ICF) promotes an in vitro mineralization in human periodontal ligament cells. The present study investigated the effect of ICF on dentin matrix protein-1 (DMP1) expression in human periodontal ligament stem cells (hPDLSCs).

Methods: Cells were treated with ICF in a serum-free culture medium for 24 h The mRNA and protein expression were examined using real-time polymerase chain reaction, immunofluorescence staining and western blot analysis, respectively.

Results: The exposure to ICF in a serum-free condition significantly induced DMP1 expression in both mRNA and protein levels. The effect of ICF-induced *DMP1* expression was inhibited by pretreatment with cycloheximide, indicating the requirement of the intermediated molecule(s). Pretreatment with transforming growth factor β (TGF-β) receptor inhibitor (SB431542) or neutralized antibody against TGF-β1 prior to ICF application abolished the effect of ICF-induced *DMP1* expression. Further, recombinant TGF-β1 treatment stimulated *DMP1* expression.

Conclusion: The present study illustrated that ICF induces DMP1 expression in hPDLSCs via the regulation of TGF-β signaling pathway.

Keywords: Compressive force, Dentin matrix protein, Periodontal ligament, Transforming growth factor

PA-00002

Formulation of 1% α Mangostin in Orabase Gel Induces Apoptosis in Oral Squamous Cell Carcinoma

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Poster Presentation

ABSTRACT

Background and Objectives: Plant derived compounds have chemopreventive properties which may be used as an alternative method in preventing or delaying tumor progression. The mangosteen pericarp derived xanthones, α mangostin(α MG) is the most abundant phytochemical that demonstrates potent anticancer effects against various types of cancer. The objectives of this study were to develop oral gel formulations containing α MG and determine their 1) anticancer activity, 2) anti HPV 16 and antimicrobial activities, 3) nitric oxide (NO) inhibitory activity, and 4) wound healing effect.

Methods: Formulations of oral gel containing α MG were developed. Anticancer activity on SCC 25 was assessed. Apoptotic induction was determined using flow cytometry technique. Antiviral activity against HPV 16 pseudovirus and antimicrobial activity against *S. mutans*, *P.gingivalis* and *C.albicans* were investigated. Nitric oxide (NO) inhibition was carried out. Fibroblast cell migration was determined by *in vitro* scratch assay.

Results: The formulation of 1% α MG in orabase gel demonstrated anticancer activity by promoting apoptosis in SCC-25. The induction of apoptotic activity was dose dependent with pronounced effect in late apoptosis. The formulation appeared to reduce cell viability of oral keratinocytes (OKC). At CC50 it showed an inhibition against HPV-16 pseudovirus infection. The formulation had no antimicrobial activity against *S. mutans*, *P. gingivalis* and *C. albicans*. No significant NO inhibitory activity and wound healing effects were found.

Conclusion: The formulation of 1% α MG in orabase gel demonstrated anticancer activity by inducing apoptosis. However, it affected viability of OKC. Appropriate carrier of novel nanoparticles targeting cancer cells should be further investigated.

Keywords: Apoptosis, α-mangostin, Orabase gel, Oral cancer, Oral squamous cell carcinoma

PA-00004

Effect of Adhesive Removing Methods on Shear Bond Strength of Reused Ceramic Brackets

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Poster Presentation

ABSTRACT

Background: The ceramic bracket is widely used due to aesthetics concerned during orthodontics treatment. The unintentional dislodgement during the treatment causes orthodontists to consider bonding a new bracket or reusing an old bracket. Due to the high-priced of a new ceramic bracket, reusing an old bracket is a proper selection. Before reattachment the old bracket, removing adhesive remnants method on bracket base should be performed. The efficiently method will provide the great bonding properties.

Objective: This study aimed to compare the shear bond strength of reused ceramic brackets after removing adhesive remnants using different chairside methods; sandblasting, greenstone bur, tungsten carbide bur, and ultrasonic scaler.

Methods: Thirty ceramic brackets were divided into five groups; one group of new brackets for control and four groups of reused brackets which are bonded and debonded then removing adhesive remnants with the different methods; sandblasting, greenstone bur, tungsten carbide bur, and ultrasonic scaler. After that, the brackets of all groups were attached to the upper first premolar teeth using adhesive. The shear force was measured by universal testing machine then calculated into shear bond strength.

Results: The shear bond strength of reused brackets after sandblasting group presented no significant difference with the new bracket group ($p>0.05$).Moreover, the shear bond strength of both groups are significant greater than other ($p<0.05$).

Conclusion: Sandblasting is suitable for removing adhesive remnants on reused bracket, as it showed efficacy to remove adhesive remnants on bracket base while maintaining adequate shear bond strength as the new bracket.

Keywords: Adhesive, Bracket, Orthodontics, Recycled, Reused

PA-00005

A Study of Suitable Thymol Solubility for use in Dentistry

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Poster Presentation

ABSTRACT

Background and Objectives: Thymol (2-isopropyl-5-methylphenol) has a broad spectrum antimicrobial effect. According to its biological properties, Faculty of Dentistry at Mahidol University has developed 0.1% w/v thymol product for storaging tooth specimens for dental research. However, thymol is slightly soluble in water, which makes several days for a preparation of 0.1% w/v thymol in the water. As a result, a shortage of the thymol product usually occurs. Therefore, this research aimed to reduce a preparation time of the 0.1% w/v thymol product, while preserve the same active ingredients and its properties by using ethyl alcohol. Our study determined the appropriate concentration of the thymol solvent and the concentration of thymol after dissolution.

Methods: The preparation time and the minimum volume of various alcohol-based solvent such as 55%, 75%, 95% and absolute alcohol used for dissolving thymol were determined. Then, the active compound of thymol solution was determined by HPLC analysis method.

Results: The results showed that the minimum volume of various alcohol concentrations was 3 ml per total volume 100 ml to dissolve the thymol completely. Furthermore, HPLC analysis demonstrated 0.079, 0.089, 0.087, 0.087 and 0.086 %w/v thymol was found in the prepared solution using deionized water, 55%, 75% 95% and Absolute alcohol, respectively.

Conclusion: In conclusion, alcohol is a good solvent for preparing thymol product the amount of thymol in 0.1% thymol solution in various alcohol-based solvents was higher than water dissolve. This method of preparation can be used for further development for other studies.

Keywords: Thymol, Dissolution, Dentistry

PA-00006

Effect of Post-Rinsing Time and Method on Accuracy of Stereolithography Photopolymer Resin

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Poster Presentation

ABSTRACT

Objective: This in vitro study investigates the effect of different post-rinsing times and methods on the trueness and precision of denture base resin manufactured through stereolithography.

Methods: Ninety clear photopolymer resin specimens were fabricated and divided into nine groups (n=10) based on rinsing times and methods. All specimens were rinsed with 99% isopropanol alcohol for 5, 10, and 15 min using three methods-automated, ultrasonic cleaning, and hand washing. The specimens were polymerized for 30 min at 40°C. For trueness, the scanned intaglio surface of each SLA denture base was superimposed on the original standard tessellation language (STL) file using bestfit alignment (n=10). For precision, the scanned intaglio surface of the STL file in each specimen group was superimposed across each specimen (n=45). The root mean square error (RMSE) was measured, and the data were analyzed statistically through one-way ANOVA and Tukey test (α <.05).

Results: The 10-min automated group exhibited the lowest RMSE. For trueness, this was significantly different from specimens in the 5-min hand-washed group (P <.05). For precision, this was significantly different from those of other groups (P <.05), except for the 15-min automated and 15-min ultrasonic groups. The color map results indicated that the 10-min automated method exhibited the most uniform distribution of the intaglio surface adaptation.

Conclusion: The optimal postprocessing rinsing times and methods for achieving clear photopolymer resin were found to be the automated method with rinsing times of 10 and 15 min, and the ultrasonic method with a rinsing time of 15 min.

Keywords: Additive manufacturing, Stereolithography, Trueness, Precision, Post rinsing

PA-00007

Amelogenesis Imperfecta: Tooth Characteristics in Thai Families

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Poster Presentation

ABSTRACT

Background: Enamel is a strong barrier protecting the teeth from physical, thermal and chemical stimuli. However, some hereditary conditions affect the quantity and quality of enamel and compromise esthetics and functions. Amelogenesis imperfecta (AI) is a rare genetic disorder that alters the enamel formation of both primary and permanent dentitions. Clinical features of AI are categorized into hypomaturation, hypocalcification (hypomineralization), and hypoplastic. At present, the understandings of AI tooth defects are still unclear.

Objective: To investigate oro-dental structures of three patients affected with hypoplastic-hypocalcified AI.

Materials and Methods: Tooth color, roughness, mineral density, mineral content, and ultrastructure of AI teeth obtained from three affected individuals were examined and compared with the control teeth obtained from healthy individuals.

Results: The color of AI teeth ranged from white-yellowish to brown. AI1 and AI2 revealed significant increase in surface roughness while AI3 had decreased roughness. All AI teeth showed significant decreases in enamel mineral density. Calcium and phosphorus ratios were increased in AI2 and AI3 enamel as well as in the dentin of all AI teeth. Remarkably, enamel rods were severely collapsed and a gap of dentinoenamel junction was presented in all AI teeth, suggesting enamel-dentin discontinuation.

Conclusion: Hypoplastic-hypocalcified AI teeth demonstrated abnormalities in color, surface roughness, mineral density, mineral composition, and ultrastructure. This study expands an understanding of AI tooth defects.

Keywords: Enamel, Mineral density, Roughness, Ultrastructure

PA-00008

Factors Influencing the Dietary Behavioral of Preteenagers in Bangkok, Thailand

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ABSTRACT

Background and Objectives: Many researches have shown the immense problems of preteenagers' oral health conditions which comprise a strong foundation for health throughout life is eating habits. This cross-sectional descriptive research aims to investigate the knowledge of oral disease, nutrition, oral care and regular dental visits and individual beliefs; perceive severity of disease, perceived susceptibility to disease, perceived benefits of prevention action, perceived barrier preventive action and perceived self-efficacy influencing the dietary behavioral of 12 years old children, primary school in Wattana province, Bangkok.

Methods: The closed ended questionnaires and the rating- scale questionnaires with 0.72-0.85 credibility level was used for data collection. The knowledge and the individual beliefs were assessed using dietary behavior counts as the dependent variables in 335 children. The descriptive statistics, One-way ANOVA and Pearson's correlation were applied.

Results: Of the 335, 12-year-old students, 310 (92.5 %) provided questionnaire data. There is positive correlation with dietary behavior which was knowledge of nutrition (r=0.33) and the individual beliefs which was the perceived of self-efficacy (r=0.19).

Conclusion: Dietary behavior of primary school children, which associated with their oral health, has found to be significantly related to knowledge of nutrition and the perceived self-efficacy (r=0.33 and 0.19, p value< 0.05)

Implications: The findings of this study will assist policy makers by highlighting the individual believe in preteenagers that should be part of future health promotion programs.

Keywords: Dietary behaviors, Food consumption, Oral health, Preteenagers, Self-efficacy

PA-00009

Cytotoxicity of Newly formulated Chlorhexidine mouthwash against Mouse Fibroblasts

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Poster Presentation

ABSTRACT

Background and Objectives: The new formula Chlorhexidine possesses the same level of antimicrobial properties as before. However, in regards to safety, a standardized toxicity test must be carried out for its further development. To evaluate the cytotoxic effects of newly formulated chlorhexidine mouthwash on mouse fibroblasts cells.

Methods: Chlorhexidine mouthwash was prepared in the laboratory according to the original formula (FDMU). Inoculums of L929 cell line (cell density 1x105 cell/well) were prepared in 96-well cell culture plates and incubated in a CO_2 incubator at 37°C with a relative humidity of 95% for 24 hrs. The toxicity test was performed by using the MTT assay.

Results: Cytotoxicity assessment on L929 mouse fibroblasts cell line revealed that the % cell viability was the highest with chlorhexidine gluconate-new formulation, followed by commercially available, and original formula MU Dent chlorhexidine gluconate (% cell viability = 41.50, 41.21, and 39.19, respectively).

Conclusion: The newly developed chlorhexidine mouthwash can be considered as safe for oral usage. However, a user satisfaction test must be conducted regarding its palatability and after-taste.

Keywords: Cell viability, Chlorhexidine, Cytotoxicity, L929, Mouthwash

PA-00010

Sex and Age Classification from Radiographic Femur using Machine Learning Algorithms: A Preliminary Study

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Poster Presentation

ABSTRACT

Background and Objectives: Femur is one of the pragmatic traces for age and sex identification in cases where maceration of soft tissues is not advised, or other indicators are absent. We aimed to evaluate performances of machine learning (ML) algorithms for sex and age classification from radiographic femurs.

Methods: We acquired radiographs of 354 left proximal femora of which were documented sex (193 males and 161 females) and age (range = 19-94 years). We measured dimensions and quantified bone density at the femoral neck, ward's triangle, and greater trochanter using the ImageJ program. The dimensions indicated sex and trabecular density represented age. We quantified pixel density of trabecular structure of each femur by applying Otsu's threshold. Separate age estimation models were formulated for each sex. All parameters in Excel format were fed to train and validate the ML algorithms using MATLAB programming software.

Results: Using 5-fold cross-validation, K-nearest neighbor (KNN) model showed the highest accuracy of 92.4% in sex estimation, albeit slightly better than the optimizable discriminant and support-vector machine algorithms (accuracy = 92.1% and 91.5%, respectively). In male samples, the top-performing algorithm in age estimation is linear regression with root mean square error (RMSE) of 12.813. Gaussian process regression revealed RMSE of 12.829 in female dataset.

Conclusion: KNN can be used to estimate sex from radiographic femur with high accuracy. However, ML algorithms performed unremarkably in age prediction. Trabecular density might not correlate with age. Further study is strongly encouraged to verify objectively assess fragmentary remains.

Keywords: Age estimation, Forensic anthropology, Machine learning, Proximal femur, Sex estimation

PA-00011

Antimicrobial action Newly Formulated Chlorhexidine Mouthwash against Oral Pathogens

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Poster Presentation

ABSTRACT

Background and Objective: Chlorhexidine gluconate is classified as a disinfectant and is effective against a wide range of microorganisms. It is also popularly used as a topical solution in dentistry. However, chlorhexidine gluconate mouthwash has a poor taste during and after rinsing which makes its users dissatisfied. Therefore, the product must be improved to have a better taste but still retain its effectiveness against oral pathogens. To evaluate in vitro antimicrobial activity of modified chlorhexidine mouthwash against oral microorganisms.

Methods: Chlorhexidine mouthwash new formulation was prepared in the laboratory based on the original formula (Faculty of Dentistry, Mahidol University) and was evaluated for the antimicrobial properties. The microorganism used in the study were Streptococcus mutans, Streptococcus pyogenes, Lactobacillus casei, and Candida albicans. The antimicrobial activity of the mouthwash was evaluated by agar disc diffusion method against the tested microorganisms. Distilled water was used as the negative control. 0.12 % Chlorhexidine diluted served as positive control.

Results: The newly formulated chlorhexidine mouthwash exhibited antimicrobial activity against all microorganisms with inhibition zones ranging from 17-19 mm, whereas the inhibition zone diameter of negative control was 0 mm.

Conclusion: Data from our study showed that the newly formulated chlorhexidine mouthwash retained its effectiveness against Streptococcus mutans, Streptococcus pyogenes, Lactobacillus casei, and Candida albicans. The new formula has the potential to treat and prevent oral and throat infections.

Keywords: Antimicrobial activity, Chlorhexidine, Microorganisms, Mouthwash, Oral pathogen

PA-00012

Comparative study of Reciprocating NiTi File Systems in Preparing S-shaped Canals

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ABSTRACT

Background and Objectives: Thermomechanical treatment of NiTi alloy and reciprocating movement of the NiTi rotary files were proved to reduce the risk of procedural errors during root canal preparation.The purpose of this study was to compare the amount of canal deviation after root canal preparation with WaveOne Gold, Reciproc Blue, and EdgeOne Fire reciprocating file systems in simulated S-shaped canals.

Methods: The clear resin blocks were used to simulate the S-shaped root canals. There were 7 blocks for each group which were prepared by one of the three file systems. The pictures of canal were taken before and after root canal preparation. The pre- and post-instrumentation images were superimposed using the ImageJ program and the amount of resin removed was evaluated. The data was analyzed using one-way ANOVA and Tukey's HSD test at $P<0.05$.

Results: The direction of canal deviation was toward the inner side of curve at every level of all groups. At coronal curvature, the higher distances of canal transportation were found in the Reciproc Blue group. At the apical curvature, WaveOne Gold and Reciproc Blue showed the comparative amount of resin removal which was more than EdgeOne Fire group ($P<0.01$). However, at the end of apical curvature level, the EdgeOne Fire group revealed the highest distance of canal transportation ($P<0.01$).

Conclusion: Under the condition of this study, canal deviation was minimized when WaveOne Gold NiTi system was used in S-shaped root canals.

Keywords: Canal deviation, S-shaped root canal, Nickel-titanium file, Reciprocating file, Single file

Poster Presentation

PA-00014

The Trend of Artificial Intelligence Publications in Dentistry

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Poster Presentation

ABSTRACT

Background and Objective: Artificial Intelligence is a technology applied in the field of dentistry for diagnosis, treatment plan and various predictions in dentistry. The study of Artificial Intelligence research direction in international published journals is important because it is an indicator of technology direction and benefits to be information for researchers to R&D in dental technology.

Methods: The unit of analysis was composed of each dentistry publication that appears in the Scopus database. Since our software (Scopus) can analyze the last decade, the study period was limited from 2012 to 2021. The publications were screened and examined based on the title and abstract.

Results: The total search of Artificial Intelligence Publications in Dentistry was 1,322 manuscripts. Studies included in quantitative synthesis were 324 manuscripts. Our statistics demonstrated an increasing trend in publications during the past four years, significant growth in published was observed, with 11 manuscripts in 2018 and 190 manuscripts in 2021. An increasing trend of countries in publications during the past four years, significant growth in published was observes.

Conclusion: Studies have shown that artificial intelligence in dentistry is trending between 2017 and 2021 for use in dental diagnosis and treatment planning. Leading countries were India, the United States, and China, respectively, with the title Artificial intelligence for automatic prediction of required surface roughness by monitoring wear on face mill teeth cited the most, 110 times.

Keywords: Artificial Intelligence, Dentistry, Publications, Technology, Trend

PA-00015

Effect of *Kaempferia parviflora* Extract on Proliferation and Migration of Human Gingival Fibroblasts

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ABSTRACT

Background and Objectives: Wound healing is a repairing process of damaged tissues where fibroblasts serves as key players to promote wound closure and extracellular matrix remodeling. *Kaempferia parviflora* is a traditional herb which have been reported to have anti-mycobacterial and anti-inflammatory properties. However, the roles of *K. parviflora* in oral wound healing remain to be explored. Therefore, this study aimed to investigate the in vitro effect of *K. parviflora* on cell proliferation and migration of human gingival fibroblasts (HGFs).

Methods: The effect of *K. parviflora* ethanolic extract at different concentrations (0.46, 0.94, 1.87, 3.75, and 7.5 mg/ml) on HGFs cell viability and proliferation was determined by MTT assay at 1, 3, 5, and 7 days. Cell migration was then monitored by a scratch assay at 12, 24, 48 hours after *K. parviflora* treatment.

Results: The results showed that *K. parviflora* at 0.46 mg/ml did not decrease cell viability of HGFs at all experimental time points and significantly increased cell proliferation at day 3, 5, and 7 as well as cell migration at 24 and 48 hours compared to the control. Conversely, the extract at higher concentrations decreased cell viability and cell migration in dose- and time-dependent manners.

Conclusion: *K. Parviflora* extract at low concentration (0.46 mg/ml) had no cytotoxic effect and could promote proliferation and migration of HGFs to the wound area. This finding suggested that *K. Parviflora* extract can be a potential candidate for future development of the oral wound care product.

Keywords: Cell migration, Cell proliferation, Cytotoxicity, Human gingival fibroblast, *Kaempferia parviflora*

PA-00016

Antibacterial Activities of Thai Medicinal Herbal Extracts Against Dental Pathogens

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Poster Presentation

ABSTRACT

Background: Dental caries are one of the most common oral infectious diseases. The interactions among bacteria, host, diet, and time can result to tooth decay. *Streptococcus mutans*, *Enterococcus faecalis*, and *Lactobacillus casei* are bacteria related to dental caries. *Streptococcus mutans* is the main etiologic agent of dental caries. Three Thai medicinal herbal (*Clinacanthus nutans*, *Piper nigrum* and *Curcuma longa linn*) had been found to have antibacterial acitivity.

Objectives: The aim of this study was to evaluate the antibacterial activities of extracts from three Thai medicinal plants (*P. nigrum*, *C. nutans*, and *C. longa linn*.)

Methods: Three bacterial *S mutans, E. faecalis,* and *L. casei* were used in the antimicrobial study employing minimal inhibitory concentration (MICs).

Results: The Piper nigrum seed extract gave zones of inhibition of 34.4, 25.0, and 14.9 mm against *S. mutans*, *L. casei*, and *E. faecalis*, respectively. The *C. nutans* extract gave zones of inhibition of 38.6, 24.8, and 14.9 mm against *S. mutans, L. casei* and *E. faecalis*, respectively. *C. longa linn* extract gave zones of inhibition of 33.5, 26.5, and 14.9 mm against *S. mutans, L. casei*, and *E. faecalis*, respectively. The positive control gave a zone of inhibition of 18.3-18.6 mm for all 3 studied bacteria.

Conclusion: *C. nutans*, could effectively inhibit the growth of *E. faecalis, L. casei*, and *S. mutans*. Although the three Thai medicinal plants had antibacterial activities against the three bacterial strains with MICs ranging from 0.95 to 31.25 µg/ml, the those three bacteria and whether they can prevent dental caries and periodontal diseases.

Keywords: Antibacterial activity, Herbal extracts, *Piper nigrum*, *Clinacanthus nutans*, *Curcuma longa linn*

PA-00018

Effect of Different Microcrystalline and Paraffin Ratio on the Volumetric Shrinkage of Dental Wax

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Poster Presentation

ABSTRACT

Background and Objectives: Volumetric shrinkage is the main problem of dental wax used in dental works. It affects on quality of work, especially in dental prosthetic process. Dental wax mainly consists of paraffin, which has influence on volumetric shrinkage property, while microcrystalline can possibly reduce volumetric shrinkage. Therefore, this study aimed at experimenting the proper microcrystalline and paraffin ratio in dental wax on reducing volumetric shrinkage and following the standard testing of ISO 15854 and ADA specification no.24.

Methods: Microcrystalline was added into dental wax at five different ratios, 1:99, 2:98, 3:97, 4:96, 5:95. Volumetric shrinkage in each sample and pure paraffin was measured by Archimedes' principle using Eureka can. Flow property, behavior on softening, appearance after flaming, residue on artificial teeth, and adhesion on storage were also tested following ISO 15854 and ADA specification no.24 guidelines.

Results: Volumetric shrinkage of each ratio, 1:99, 2:98, 3:97, 4:96, 5:95, was 11.56%, 10.63%, 7.81%, 10.63%, 13.13% compared to 13.43% of pure paraffin. All ratios passed all properties of ISO 15854 and ADA specification no.24 except flow property.

Conclusion: Microcrystalline and paraffin ratio which reduced volumetric shrinkage the most found in this experiment was 3:97. Flow was the only property which did not pass ISO 15854 and ADA specification no.24.

Keywords: Dental wax, Paraffin, Microcrystalline, Volumetric shrinkage, Flowing property

PA-00019

Effect of Nicotine on Primary Mouse Embryonic Fibroblasts *in vitro*

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Poster Presentation

ABSTRACT

Background and Objectives: Nicotine found in tobacco has cytotoxic effect on fetal development. The research of epidemiology found the association between maternal smoking and oral clefts statistically signification. However, the underlying mechanisms have not been clarified. To give some insight into the cytotoxic effect of nicotine on mesenchymal cells developing in palate formation. Primary mouse embryonic fibroblasts (PMEF cells) representing the mesenchymal cells comparable to critical stage of palate formation (E13) was chosen.

Methods: PMEF cells were cultured and treated with 3, 4, 5 mM nicotine. MTT assay was used to detect cell viability. Morphological change was identified by fluorescence microscope and the quantity of cell apoptosis was revealed with HT Titer TACSTM. The generation of ROS was assessed using ROS-Glo H2O2 Assay. RT-qPCR was performed to verify the expression of BAX, BCL2, Caspase3 and P53 genes.

Results: Results revealed that nicotine decreased cell viability, increased apoptosis cell death and ROS in a dose dependent-manner. Nicotine at 5 mM increased expression of BAX, BCL2, Caspase3 and P53 comparison to that of the untreated control.

Conclusion: Nicotine had an adverse effect of PMEF cells by increase the generation of ROS. The ROS overproduction was closely associated with quantity of cell viability and cell apoptosis and expression of apoptosis-related genes such as CAS3 and P53, which lead to cell apoptosis.

Keywords: Apoptosis, Nicotine, Reactive oxygen species, PMEF cells, P53

PA-00020

Generation Gap Project: An Application Health Promotion Concept for Dental Students

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ABSTRACT

Background and Objective: The generation gap is a crucial determinant of health in organizations and society. This study aimed to prepare dental students to deal with this issue in their future work life based on the health promotion concept.

Methods: Project-based learning was developed for the 8 fifth-year dental students in the Community dentistry practice from May 17 to June 21, 2022. The learning process followed the project cycle to implement the health-promoting project with the issue of a generation gap in 24 back-office employees who were of different ages at the faculty of dentistry, Chiang Mai University. Two teachers were facilitators throughout the learning process.

Results: The students could apply the health promotion concept into practice. In the situation analysis, they could review the literature and interview people in varied age groups to understand the gap between generations from various points of view. In the planning, after data analysis and considerable discussion, the students created the idea to do a workshop to enhance an understanding of the generation gap. To gain participation, they worked with 4 heads of back-office to finish the final plan. For implementation, 24 back-office employees were asked to join the workshop. For evaluation, Questionnaires and self-reflection were used. In addition, students could apply the health promotion concept into practice and recognize the generation gap issue shown by self-reflection VDO clips.

Conclusion: This learning experience based on the application of the health promotion concept will help the students deal with future gap generations.

Keywords: Health promotion, Dental students, Dental education, Generation gap

PA-00021

Bond Strength Between Orthodontic Metallic Brackets Bonded to Different Tooth-colored Restorative Material

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Poster Presentation

ABSTRACT

Background and Objectives: Due to the high demand of esthetic dentistry has increased and the longevity and quality of bond between orthodontic bracket and tooth restoration are one of the most important factors of orthodontic treatment. This study aimed to compare the shear bond strength of orthodontic metallic brackets bonded to different tooth-colored restorative materials and the surface roughness of the restorations before and after bonding.

Methods: The 50 specimens (N=50) were divided into 5 groups, including resin composite (RC), Porcelain (PL), Lithium disilicate glass (LD), Zirconia (Zr), and Hybrid ceramic (HC), each of 10 (n=10). Specimens were bonded to orthodontic metallic bracket using the same cement. Shear bond strength, adhesive remnant index and surface roughness value data were collected and analyzed by One-Way ANOVA and Turkey test ($p<0.05$), Chi square, and paired T-test ($p<0.05$) respectively.

Results: The highest shear bond strength was PL group (6.24 MPa) and the lowest was RC group (3.23 MPa). RC and PL have significantly difference of shear bond strength. According to Mode of ARI scores, most groups showed score 3, except RC group which showed score 1. Surface roughness value of HC group before and after polishing is the only group that is not significantly different. ($p < 0.05$).

Conclusion: PL and ZR show the adequate shear bond strength for orthodontic bonding. Every group, except RC, has adhesive failure at adhesive-bracket interface. HC is the only group that doesn't have significantly different roughness values after polishing.

Keywords: Orthodontic metallic brackets, tooth-colored restoration, Shear Bond Strength (SBS), Adhesive Remnant Index (ARI), surface roughness value (Ra)

PA-00022

Effect of Oxidation Heat Treatment on Oxide Layer Thickness and Bonding Strength of Reused Co-Cr Alloy for PFM

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ABSTRACT

Background and Objectives: The purpose of this study is to evaluate the effect of different oxidation heat treatment (OHT) on the oxide layer thickness and bonding strength between ceramic recasting Cobol–chromium (Co-Cr) alloys.

Methods: Ninety specimens with the dimension of 15.0±0.2 mm. x 15.0±0.2 mm. x 3.0±0.1 mm. were used to evaluate the effect on the Oxide layer thickness and the other ninety plates with the dimension of 25.0±0.1 mm X 3.0±0.1 mm X 0.5±0.05 mm were used to evaluate the effect on the bonding strength, according to the ISO 9693 standard (Dentistry–compatibility Part 1: Metal–ceramic system). For each OHT method, the specimens were divided into 18 groups: 790°C, 890°C and 980°C for 30s, 60s, 90s, 120s, 150s and 180s, respectively. The Oxide layer thickness and elemental composition were analyzed using a scanning electron microscope (SEM) and Energy Dispersive X-ray Spectroscopy (EDS).

Results: The results of oxide thickness and oxygen concentration were presented in the supplementary oxides on the surface varied according to the different temperature and time duration in the following order: OHT at 980°C > OHT at 890°C > at 750°C and longer duration > shorter duration. A micro crystal structure of surface topography was found in 150s, 180s of 790°C group and all duration of 890°C group.

Conclusion: In this study, the bonding strength of all experimental group exceeds the minimum acceptable value of 25 MPa recommended by ISO 9693. Therefore, the reused Co-Cr dental alloy fabricated with these OHT techniques could be a capable alternative for metal ceramic restorations.

Keywords: Co-Cr alloy, Oxidation, Oxide layer, Bond strength, PFM

PA-00023

Effect of Herbal Extract Toothpaste on Oral Malodor Reduction

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Poster Presentation

ABSTRACT

Background and Objective: One out of four people suffers from persistent bad breath. Oral malodor is the result of the degradation of organic substrates by anaerobic bacteria of the oral cavity. The management is done by eliminating the cause (bacteria and their substrates) either mechanically or chemically. Toothpaste have been modified to carry antimicrobial with an impact on the process of oral malodor formation. The herbal extracts and essential oils demonstrated to be active against halitosis. In addition to antimicrobial efficacy of plant extracts, their aromatic effects are appreciable for bad breath neutralization and flavoring the treatment products as well. This study aimed to investigate the effect of toothpaste containing herbal extracts on the reduction of volatile oral malodor gase.

Methods: Twenty subjects with good health were recuited. They were randomly assigned herb extract toothpaste; formulation A and formulation B with moisturizer agent. The assessment of malodor measured the quantity of oral malodor gas by using an OralChroma™ device. The gas collection was collected into seven time periods; baseline (before breakfast and morning oral hygiene) and after hygiene 1, 2, 3, 6, 8 and 12 hours.

Results: The baseline mean of total VSC in toothpaste formulation A and B was 238.50±78.49 and 220.75±49.83 ng/10mL. The concentration of H_2S, CH_3SH and total VSCs at 1, 2, 3, 6 and 8 hours was significant reduced in all formulation. At 12 hours after treatment, the concentration of H_2S, CH_3SH and total VSCs was statistically significant different between formulation A and formulation B.

Conclusion: Herbal toothpaste demonstrated to significantly reduce the oral malodor in 8 hours after treatment.

Keywords: Oral malodor, volatile sulphur compound, Herbal extracts, Toothpaste, Halitosis

PA-00024

Wear of Pit and Fissure Sealants after Chewing Simulation

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ABSTRACT

Background and Objective: Abrasive wear is one of the most common types of wear that not only affects the teeth but also the dental restorations such as pit and fissure sealant. This study aimed to evaluate the abrasive wear of pit and fissure sealants by measuring volume loss, mean depth, and surface roughness after chewing simulation.

Methods: The specimens were prepared in metal molds size 10 mm in diameter and 2 mm in height and divided into three groups (n=10); Clinpro Opaque (3M ESPE, USA), Teethmate Opaque (Kuraray, USA), and Teethmate Clear (Kuraray, USA). The specimens were subjected to 120,000, 240,000, and 360,000 strokes at a load of 50 N with 1 mm lateral movement using a 6 mm diameter steatite ball as an antagonist on the Chewing Simulator (CS-4, SD Machatronik, Germany). The volume loss, mean depth, and surface roughness were determined using the Profilometer (TalyScan 150, Taylor Hobson Ltd., England) after chewing cycles by scanning in the area of 5 x 5 mm.

Results: Clinpro Opaque showed the highest volume loss and mean depth while Teethmate Opaque revealed the lowest volume loss and mean depth after chewing simulation when compared at the same chewing cycles. Clinpro Opaque also showed the highest surface roughness at 120,000 cycles among all groups. However, the surface roughness was not significantly different among all groups at 240,000 and 360,000 cycles.

Conclusion: Clinpro Opaque showed the highest volume loss, mean depth, and surface roughness after chewing simulation.

Keywords: Chewing simulation, Microhardness, Pit and fissure sealant, Wear

PA-00025

Study of Oxidation Behavior on Utilized Co-Cr Alloy Specimens for PFM

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ABSTRACT

Background and Objective: There is a shortage of dental scientific literature. Regarding the difference in OHT affecting the weight change between base alloy and porcelain. The purpose of this study was to analyze the effect of different times and temperatures for oxidation samples of Co-Cr alloys that were recycled and used for porcelain fused to metal (PFM) restorations.

Methods: The comparative high temperature oxidation rate studies were carried out using different reused of Co-Cr alloys with different temperatures of 790°C, 890°C and 980°C and different times of 30, 60, 90, 120, 150 and 180 second that were used for oxidation. The kinetics of oxidation rate of Co-Cr alloys were established with weight change measurements. The decrease of weight related to the percentages of the reused alloys 's compositions used for calculation of the oxidation rate.

Results: The least weight change per unit 1.25068 ug/mm^2 of surface area group belonged to the group with the shortest sintering time and lowest sintering temperature (790°C and 30 second). The greatest weight change 1.76000 ug/mm^2 was found in the group with the highest sintering temperature (980°C) and longest sintering time (180 s). It seemed that the kinetics were parabolic for all conditions.

Conclusion: The wight change of all experimental groups exceeded the lowest change in the group sintering at 790°C at 30 s.

Keywords: Co-Cr alloy, Oxidation, Weight change

PA-00026

Blending Effect of Resin Composites with Surrounding Structures

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ABSTRACT

Background and Objectives: Some resin composites possess the ability of blending their color to the surrounding tooth structure. This study evaluated the blending effect of four resin composites in different depths of cavities prepared in control shade (B2) of resin composite discs.

Methods: Dual-shade resin composite specimens (DS) were made with an outer control-shade and inner hole filled with 1-, 2- or 3-mm. depths of inner test-shade. Single-shade resin composite specimens (SS) of all shades were also made with corresponding depths. An area in the center of specimen (P0mm) and an area 1 mm away from this point (P1mm) were used for the color evaluation. The color differences between test shade in SS and control shade in SS (ΔE*1) and the test shade in DS and control shade in SS (ΔE*2) were evaluated. The blending effect of test materials was the value of Color Adjustment Potential (CAP = 1- (ΔE*2/ ΔE*1).

Results: For most test specimens at 1 mm depth, ΔE*2 were higher than ΔE*1. These differences decreased when materials depth increased. The ΔE*2 at P0mm were higher than those of P1mm in most test materials in deeper cavities. The CAP of evaluated shade ranged from -5.85 to 0.68. Most tested materials showed positive CAP at the depth of 3 mm. The highest CAP was recorded for Estelite Sigma, followed by Beautifil II, Ceram X SphereTEC One and Filtek Z350XT, respectively.

Conclusion: The majority of test materials exhibited blending effect which was increasing with an increase in restoration depth.

Keywords: Blending effect, Color adjustment potential, Resin composite

PA-00027

The Effect of Xylitol in the Presence of Sucrose on *Streptococcus sanguinis*

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Poster Presentation

ABSTRACT

Background and Objective: *Streptococcus sanguinis* is an early colonizer that is commonly found in healthy dental plaque. Xylitol is known to decrease acid production and biofilm formation (cariogenic virulence) of *Streptococcus mutans*, however, there is little information on its effects on early colonizers. In addition, sucrose is present in most foods and beverages, making the use of xylitol as the sole sweetener difficult. Therefore, the aim of this study was to determine the effect of xylitol with and without the presence of sucrose on acid production and biofilm formation of *S.sanguinis*.

Methods: *S. sanguinis* was cultured for 36 h in Brain-Heart-Infusion broth (BHI) containing 1% sucrose (1S), BHI containing 1% or 10% xylitol with or without 1% sucrose (1X+1S, 10X+1S, 1X, and 10X), and BHI. To examine acid production and biofilm formation, the pH and biofilm mass (crystal violet assay) were measured and analyzed by Kruskal-Wallis with Dunn's multiple comparisons test. The experiments were performed in duplicates and repeated three times.

Results: The pH of *S. sanguinis* grown in xylitol without sucrose was significantly higher than that in 1% sucrose ($p<0.01$). However, when bacteria were grown in 1X+1S, the pH was significantly lower than that in BHI ($p<0.05$). For biofilm formation, those cultured in xylitol developed significantly less biofilm than bacteria grown in the presence of sucrose (1S, 1X+1S and 10X+1S; $p<0.05$).

Conclusion: *S. sanguinis* acid production and biofilm formation were low in xylitol. However, when sucrose is present, even at high xylitol concentrations, its effects were not apparent.

Keywords: Biofilm, pH, *Streptococcus sanguinis*, Sucrose and Xylitol

PA-00028

Production and Characterisation of Human Tetrameric Extracellular Superoxide Dismutase

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ABSTRACT

Background and Objective: Extracellular superoxide dismutase (EcSOD) is an tetrameric antioxidant enzyme localised in the extracellular matrix (ECM). The binding of EcSOD to the ECM is critical for the functions of several organs. This enzyme is also present in cementum and dentinal tubules suggesting its roles in protecting these structures from oxidative damage. Hence, this study aimed to develop a method of EcSOD production in large scale for further application in dentistry and medicine.

Methods: Human EcSOD was overexpressed in *E. coli* strain BL21 (DE3). The purification process involved protein refolding, affinity and size-exclusion chromatography in high pH buffers. The purified EcSOD (eEcSOD) was then characterised compared to the protein purified from mammalian cells (mEcSOD). SOD activity was determined by cytochrome c assay. ECM binding ability was determined by differential scanning fluorimetry (DSF). Molecular weight of the protein was determined by small angle X-ray scattering (SAXS) technique.

Results: The yield of the purified eEcSOD was 2.5 mg per one litre of culture. The percentage of inhibition of cytochrome c reduction and the shift in the melting temperature were comparable to mEcSOD. According to SAXS data, the molecular weight of 115 kDa indicated that eEcSOD was a tetrameric form.

Conclusion: To our knowledge, this is the first study to achieve the tetrameric form of human EcSOD expressed in *E. coli*. The characteristics of the protein were confirmed by its enzyme activity, affinity for ECM and molecular weight. The production process can further be improved to obtain higher amount of protein.

Keywords: Cytochrome *c* assay, Differential scanning fluorimetry, Extracellular superoxide dismutase, Protein purification, Small angle X-ray scattering

PA-00029

Antimicrobial Effects of Bicarbonate on Cystic Fibrosis Bacteria

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Poster Presentation

ABSTRACT

Cystic fibrosis (CF) is a genetic disease caused by mutations in the gene encoding the CFTR channel. In CF, the mutations lead to Cl- and HCO_3- hyposecretion, causing dehydration, acidification, and mucus accumulation in the airways. In this situation, some bacteria, such as *P. aeruginosa* and *S. aureus*, can survive and form biofilms, which are responsible for repeated infections and inflammations, and death due to pulmonary failure. Recent evidence has shown that HCO_3- may be of clinical importance since it involves in several physiological aspects in the airways, including the innate immunity by its antimicrobial effects. However, it is unclear whether the antimicrobial effects are induced by HCO_3- *per se*, or its abilities to alkalinize and increase ionic strength. In this study, we investigated the direct effects of HCO_3- on the growth and biofilm formation of the two most common CF bacteria.

P. aeruginosa and *S. aureus* were grown in the brain-heart infusion (BHI) medium and an artificial sputum medium (ASM) containing 25 or 100 mM $NaHCO_3$ supplemented with 5% CO_2 for up to 48 hours. The spectophotrometry, colony-forming assay, flow cytometry, and biofilm-crystal violet assays were used to observe bacterial growth and biofilm formation. Results were compared to the bacteria grown in $NaHCO_3$-free control media.

The data show that HCO_3- significantly decreased viable cell counts and biofilm formation in a dose-dependent manner. These effects were due neither to extracellular alkalinization nor to altered osmolality. Our findings demonstrate that HCO_3- exerts direct antibacterial and antibiofilm effects on prevalent CF bacteria.

Keywords: Bacteria; Biofilm; Bicarbonate; Cystic fibrosis; Mucus

PA-00030

Effect of 1450 ppm Fluoride Toothpaste on Surface Microhardness of Artificial Caries

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ABSTRACT

Background and Objective: Incipient carious lesion is the early sign of demineralization of the tooth. Fluoride toothpaste is a widely used product for caries prevention and remineralization. The aim of this *in vitro* study was to compare the remineralization potential of 1000- and 1450-ppm fluoride toothpaste on artificial enamel carious lesion.

Methods: Human enamel specimens with artificial carious lesions were randomly assigned into 3 groups (n=10): 1) artificial saliva (control); 2) 1000 ppm fluoride toothpaste; 3) 1450 ppm fluoride toothpaste. The specimens were subjected to 14-day pH cycling. In fluoride toothpaste groups, specimens were immersed in fluoride toothpaste slurry for 2 minutes twice a day during the pH cycling. The surface microhardness was determined at baseline, after demineralization, and after treatment by surface microhardness testing machine. The percentage of surface microhardness recovery (%SHR) was calculated and compared between the groups.

Results: The results showed that %SHR of all fluoride toothpaste groups were increased and significantly higher than the control group ($p<0.001$). The 1450 ppm fluoride toothpaste group showed significantly higher %SHR compared to 1000 ppm fluoride toothpaste group ($p = 0.021$).

Conclusion: The use of 1450 ppm fluoride toothpaste significantly increased remineralization of artificial enamel carious lesions than the use of 1000 ppm fluoride toothpaste.

Keywords: Artificial enamel carious lesion, Fluoride, Remineralization, Surface microhardness, Toothpaste

PA-00031

Effect of Immersing Orthodontic Fixed Appliances into Toothpaste Slurry on Surface Friction

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Poster Presentation

ABSTRACT

Background and Objective: Use of fixed orthodontic appliance is getting more popular nowadays. However, it put the patient at higher risk for dental caries. The use of fluoridated toothpaste along with spit, don't rinse technic is proved to maintain higher fluoride in oral environment and effective caries prevention. Many forms of topical fluoride caused deterioration of the NiTi wire's surface texture and a concomitant increase in its friction resistance to brackets. But the effect of fluoride remaining on fixed orthodontic devices from split don't rinse brushing technic to the appliances are unknown. This study aimed to investigate the effect of fluoride toothpaste remaining on the friction and surface roughness of NiTi wire.

Methods: 54 samples (9 groups) of 0.016" x 0.022" NiTi arch wires were ligated in 0.022" slot brackets. Each group was immersed into Sensodyne© toothpaste with Fluoride or Parodontax© toothpaste with Fluoride at the concentration of 0.25 ppm or 0.5 ppm for 5 days. Sensodyne© and Parodontax© without were used for positive control and normal saline for negative control. The frictional forces were measured. Surface roughness on NiTi was detected by scanning electron microscope at 150x and 500x magnification.

Results: No significant of frictional forces among each group. (p<0.05) Roughness surface between fluoride and non-fluoride groups were similar.

Conclusion: Immersing fluoride toothpaste had no effect on the frictional force on NiTi orthodontic wire. This study may suggest the split don't rinse brushing technic to orthodontic patients.

Keywords: Fluoride, Friction, NiTi, Orthodontic, Toothpaste

PA-00032

Association between Chewing Side Preference and Orientation of Mandibular Teeth in Late Adolescence

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ABSTRACT

Background and Objective: The pattern of mandibular growth obviously influences the anteroposterior direction of the lower anterior teeth. Late mandibular growth is a result from the cephalocaudal gradient of growth which increased the mandibular growth in late adolescents. Occlusal force plays an important role for maxillofacial development both maxillary and condylar growth. Anyway, the relationships between chewing side preference and malocclusion are still unclear. This study aimed to investigate the relationship between chewing side preference and malocclusion, and to study the difference of tooth orientation between unilateral and bilateral chewing side preference.

Methods: An analytical cross-sectional study was conducted among 42 patients, aged 18 – 20 years who had at least 28 permanent teeth in clinical presence (not including third molar) and at least 30 permanent teeth in panoramic radiograph (not including upper third molars). Chewing side preference was directly observed by chewing a 1.5 g piece of Xylitol® sugar free gum mint flavor for 15 seconds. Study models were taken to analyze tooth size - arch length discrepancy, dental arch width, and overjet. Panoramic radiographs were analyzed for position and angulation of lower molars.

Results: This study showed no difference of tooth orientation and malocclusion between chewing and non-chewing preference side. The inter-canine width was significantly less in unilateral chewing side preference group compared to the bilateral chewing side preference group (p<0.05).

Conclusion: The relationship between chewing side preference and malocclusion was not found in this study. However, unilateral chewing side preference may somehow affect dental arch development and led to malocclusion.

Keywords: Chewing side preference, Malocclusion, Orthodontic

PA-00033

Comparison of Tongue Characteristics Classified According to Ultrasonographic Using K-Means Clustering Algorithm

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Poster Presentation

ABSTRACT

Background and Objective: The precise correlations among tongue function and characteristics remain unknown, and no previous studies have attempted machine learning-based classification of tongue ultrasonography findings.

Methods: This cross-sectional observational study aimed to investigate relationships among tongue characteristics and function by classifying ultrasound images of the tongue using a K-means clustering algorithm.

Results: During 2017–2018, 236 healthy older participants (mean age 70.8 ± 5.4 years) were enrolled. The optimal number of clusters determined by the elbow method was 3. After analysis of tongue thickness and echo intensity plots, tongues were classified into three groups. One-way ANOVA was used to compare tongue function, tongue pressure, and oral diadochokinesis for /ta/ and /ka/ in each group. There were significant differences in all tongue functions among the three groups. The worst function was observed in patients with the lowest values for tongue thickness and echo intensity (tongue pressure [P = 0.023], /ta/ [P = 0.007], and /ka/ [P = 0.038]).

Conclusion: Our results indicate that ultrasonographic classification of tongue characteristics using K-means clustering may aid clinicians in selecting the appropriate treatment strategy. Indeed, ultrasonography is advantageous in that it provides real-time imaging that is non-invasive, which can improve patient follow-up both in the clinic and at home.

Keywords: Artificial intelligence, Ultrasonography, Tongue, Algorithm, Dysphagia

PA-00034

Teaching Empathy Through Interactive Activities for Healthcare Students

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Poster Presentation

ABSTRACT

Background and Objective: Empathy is one of life skills that have been frequently mentioned in the 21st century and it has been recognized as an essential attribute of health professional's competency. The challenge is empathy cannot be taught by lecturing. This study aimed to make an understanding and convey the value of empathy to healthcare students through interactive activities.

Methods: The 3-hour session with five activities were created for postgraduate and undergraduate dental students and undergraduate pharmaceutical students. Firstly, students were asked to revise their own experiences about empathy by communicating with a chatbot created by instructors. Secondly, the video clip from YouTube was used to clarify the differences between empathy and sympathy. Thirdly, the students practiced giving empathy to their peers in the "think-pair-share" activity. One student told their story, and another gave empathetic responses by using the given keywords or phrases assigned by instructors. After this activity, "safe space, attentively listening, no judgment, and sincerely responding" were reflected as the main idea of empathy rather than words. Fourthly, students were asked to think and share their self-empathy in a group of 7-9 students. Lastly, Applying empathy to the doctor-patient relationship was evaluated by written assignment. The students were asked to contemplate and then write about their unexpected empathic experiences as they were doctors, patients, relatives, or observers and gave reflection.

Results: The students presented their understanding, applying, and valuing empathy during class activities and written evaluations.

Conclusion: These designed interactive activities can persuasively cultivate empathy in healthcare students.

Keywords: Doctor-patient relationship, Empathy, Healthcare student, Interactive activities

PA-00035

Characteristics and Osteoconductive Property of the Cuttlebone-derived Hydroxyapatite Particles

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Poster Presentation

ABSTRACT

Background and Objective: Synthesizing hydroxyapatite (HA) from marine wastes such as cuttle bone (CB) helps to promote the circular economy and the CB is a rich source of calcium phosphate for bone substitute syntheses. The current study aimed to synthesize the HA particles from the CB (CB-HA) and characterize their properties for a bone augmentation application.

Methods: The CB-HA was synthesized using a low temperature method. Then, morphology, mineral contents, particle size, crystalline structure and ion release of the particles were examined using scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), particle size analyzer, X-ray powder diffraction (XRD), and inductively coupled plasma optical emission spectroscopy (ICP-OES), respective. After that the direct effects of the particles on cell viability, growth, and osteogenic differentiation of the human dental pulp stem cells (hDPSCs) were investigated. The significant differences were set at $p<0.05$ (n=3-5, Mean±SD).

Results: The CB-HA particles had rod-liked morphology with the particle size 304.73 ± 4.19 nm and the calcium (Ca) and phosphorus (P) ratio (Ca/P) was 1.53. The particles released high levels of the Ca and P ions with low levels of trace elements. The CB-HA particles established a direct contact with the hDPSCs and promoted cell growth and osteogenic differentiation of the hDPSCs. Levels of alkaline phosphatase activity and osteocalcin of the hDPSCs cultured with the particles were higher than a commercial bone substitute and growth medium.

Conclusion: The CB-HA was a potential bioactive bone substitute for promoting bone regeneration and alveolar bone augmentation.

Keywords: Bone substitutes, Cuttlebone, Dental pulp stem cells, Hydroxyapatite, Osteogenic differentiation

PA-00036

Foreign Body Reaction of Dermal Fillers to The Mandible: Case Report

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ABSTRACT

Background: Injectable dermal fillers is one of the most performed procedures in the cosmetic treatment. It is leading to the possibility of unexpected findings on oral and maxillofacial images.

Objective: To present the complications of dermal filler injection to the jaw and to provide the managing of these complications.

Case report: A healthy 31-years-old Thai female was referred to Department of Radiology after a panoramic x-ray, suspected a cystic formation at the right mandible. Panoramic and periapical radiograph including CBCT images revealed multiple cysts like lesion associated with multi foci of radiopaque clusters throughout all over the mandible. Labial and buccal plates were partially resorbed and perforated. She had received multiple injection of facial dermal fillers for years. The patient underwent excisional biopsy combination with surgical removal of tooth 48. The specimen showed substances as jelly-like materials and small granules within the bone and soft tissues. Microscopically, the presence of fibrous connective tissue containing focal area of multinucleated giant cells and lymphocytes consistent with foreign body granuloma. The postoperative course was uneventful and no radiographic signs of recurrence. However, long term follow-up of the other site of the mandible is needed.

Conclusion: Although dermal fillers reportedly have a low incidence of long-term side effects, the complications from the injection of fillers was challenging in the dental practice. The dentists should be aware of the possible effects of foreign body reaction to these injectable agents beside routine oral examination.

Keywords: Radiography, Cone Beam CT, Dermal fillers, Complications

PA-00037

Autotransplantation of Premolar to Maxillary Incisor in Orthodontic Patients: A Report of 2 Cases

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ABSTRACT

The maxillary incisors are the most prone to being traumatized and susceptible of tooth loss in both children and adolescents. The treatment to replace this missing tooth has always been one of the most challenging problems for dental practitioners since the dentofacial growth was not completed. Autogenous tooth transplantation of premolar to replace the missing maxillary incisors provided predictable long-term results and had ability to assure the bone preservation during general growth, as well as the transplanted tooth can be moved by orthodontic force. Moreover, the transplanted premolar could be successfully transformed to the morphology of the missing incisor which was important since the tooth was placed in the esthetic zone.

This article presented two cases, both were demonstrated with severe maxillary teeth protrusion and had orthodontic treatment plan. Beginning with the case of a 12-year-old girl with maxillary right incisor ankylosis. Autotransplantation of maxillary left premolar was done to replace the right maxillary central incisor. The second case was a 13-year-old girl with a history of motorcycle accident and subsequent multiple anterior tooth loss. One month after orthodontic treatment, she received autotransplantation of mandibular left premolar to left central incisor. In both cases, composite resin built-up for proper morphology of central incisor and orthodontic treatment was done. The 12-year-old girl case was successful after 8 years of follow-up, and the 13-year-old case had clinical and radiographic results within normal limit, with acceptable esthetic results after 5 years of follow up.

Keywords: Autotransplantation, Central incisor, Orthodontic patient, Premolar, Tooth transplantation

PA-00038

Chitosan-Based Scaffold Incorporated with Trichostatin A for Bone Tissue Engineering

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Poster Presentation

ABSTRACT

Background and Objective: Bone tissue engineering is a complicated field requiring concerted participation of cells, scaffolds, and osteoactive molecules. Still, materials that demonstrated structural and mechanical equivalence to the bone are in search. This study aims to synthesize bone substitute material from naturally derived product, chitosan (CS), and improve their physical and biological properties by incorporating biphasic calcium phosphate (BCP) and trichostatin A (TSA), an epigenetic modifier molecule.

Methods: The scaffolds were prepared by freeze-drying technique with different amounts of BCP (0%, 10%, 20%, 40% w/w) and TSA (0, 200, 400, 800, 1600 nM). The surface morphology, in vitro degradation, compressive modulus, and in vitro biocompatibility were examined. Furthermore, the release of TSA and the ability to inhibit HDAC activity were quantified. The real-time RT-PCR was used to estimate the expression of the inflammatory-related gene and osteoblast-related genes. Finally, the scaffold was implanted into a 4 mm critical size calvarial defect of mouse.

Results: The addition of BCP improved the scaffolds' mechanical properties. Whereas the 20% BCP scaffold shows the best physical characteristic. The scaffold with 800 nM of TSA showed excellent biocompatibility and induced osteoblast-related genes expression in the cells. TSA released from the scaffold was confirmed to be biologically active. Finally, micro-CT and histology revealed that CS/BCP/TSA scaffolds could promote excellent in vivo bone regeneration.

Conclusion: The scaffold with 20% of BCP and 800 nM of TSA might be an acceptable material candidate for bone tissue engineering.

Keywords: Chitosan, Biphasic calcium phosphate, Trichostatin A, Scaffold, Bone regeneration

PA-00039

A Semi-Rigid Shell Barrier System: An Alternative System for Bone Regeneration

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Poster Presentation

ABSTRACT

Background and Objective: Nowadays, there are no materials serve all the ideal propose of bone augmentation. This study aimed to fabricate a semi-rigid shell barrier system composed of a semi-rigid shell (shell) and a membrane based on polycaprolactone (PCL) and biphasic calcium phosphate (BCP) for GBR.

Methods: A shell and membrane were fabricated from 70%PCL and 30%BCP by solvent casting technique. The materials were evaluated physical characteristics, chemical composition, mechanical properties, *in vitro* biocompatibility, and *in vivo* bone regeneration capacity compared with commercial d-PTFE. The data were evaluated by one-way ANOVA followed by Tukey's post-test and Dunnett's T3 for multiple comparisons ($p < 0.05$).

Results: The fabricated materials had rough surfaces with BCP particles on one side and the opposite side were smoother with hydrophilic on both surfaces. The pore sizes approximately 5-40 µM. The shell presented higher compressive bending strength (18.82±2.72N) than the d-PTFE membrane (4.23±0.5N). The tensile strength of the membrane (2.544±0.19MPa) was not different from the d-PTFE (2.908±0.12MPa). All materials showed biocompatibility with osteoblast and fibroblast cells. At 4 weeks and 12 weeks in rabbit's calvaria's the system demonstrated greater amount of newly form bone (24.53 ± 1.34% and 35.96 ± 1.51%) than the d-PTFE (18.69 ± 1.32% and 29.72 ± 3.00%), respectively.

Conclusion: The semi-rigid shell barrier system made of PCL and BCP demonstrated suitable physicochemical characteristics, good mechanical properties, good *in vitro* biocompatibility, and good *in vivo* bone regeneration capacity.

Keywords: Barrier membrane, Biphasic calcium phosphate, Bone regeneration, Polycaprolactone, Semi-rigid shell barrier system

PA-00040

Knowledge, Attitude, and Oral Health Care Practices of Caregivers for Bedridden Patients

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ABSTRACT

Background and Objective: Caregiver is the key person in providing oral health care to bedridden patient. Oral care knowledge, attitude and practices of a caregiver may result in oral health status of the patient. Therefore, this descriptive study aims at exploring the knowledge, attitude, and oral health care practices for bedridden patients by caregivers, and to examine the relationship among those three variables.

Methods: Study subjects were 24 caregivers in 3 Tambons, Muang, Chiang Rai who had agreed to participate in the study. Data was collected by using a questionnaire consisted of knowledge, attitude, and practices questions. Caregivers were also interviewed by using non-structural oral health care practices interview form. Descriptive analysis and Chi-square correlation test were used to analyze quantitative data. Content analysis was used to interpret interview data.

Results: The average score of knowledge is high, attitude is in good level but low in oral health care practices. Relation analysis showed attitude is significantly associated with caregivers' oral practices for bedridden patients (p-value ≤0.05). Knowledge does not correlate with oral health care practices. Other factors which contribute to caregivers' behavior on oral health care for bedridden patients, are socioeconomic status, environment, experience and time in caring patients.

Conclusion: Knowledge and attitude of caregivers in oral health care for bedridden patient is good but not for the practices. This study suggests the support and more oral care practice instruction to the caregivers.

Keywords: Knowledge, Attitude, Oral care practices, Bedridden patients, Caregivers

PA-00041

The Dilemmas of Professional Development that Dental Students Face during Clinical Practice

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Poster Presentation

ABSTRACT

Background: Professionalism development is a crucial topic in dental students curriculum of every dental school. Some studies found that experiences in professionalism dilemmas are important to individual development. On the other hand, there is a lack of studies about this topic. Objective of this research is to study through ethical dilemma experiences to develop dental professionalism.

Methods: Datas is collected from Chiang Mai University clinical dental students by writing the individual narrative of their professionalism dilemma situations via online platform. Qualitative study processes are used and datas are collected, categorized and discussed by researchers to find a conclusion.

Results: There are 43 participants (20.67%) out of 208 students. Of all participants, there are 5 fourth-year students, 20 fifth-year students and 18 sixth-year students. Narratives are categorized into 2 main themes– conflict between students' and patients' benefit (19 narratives) and improper interactions between students and instructors (16 narratives)– and other miscellaneous issues (9 narratives).

Conclusion: Clinical dental students always encounter professionalism dilemma situation as problem so these should be paid attention & should be studied for professionalism development in further.

Keywords: The Dilemmas of Professional Development , Dental students , Qualitative research

PA-00042

System Dynamics Model of SSB Tax and Dental Caries in Thai Children

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ABSTRACT

Background and Objective: Since 2017 the sugar-sweetened beverage (SSB) tax was implemented in Thailand to encourage industries to reduce sugar content in beverages. This study aims to estimate the changes of dental caries prevalence of Thai children after the implementation of SSB tax by using system dynamics modeling.

Methods: A system dynamics model was developed to capture the dynamics relationships between dental caries, sugar consumption and SSB tax policy. The model building comprised a qualitative model (causal loop diagram) of factors affecting dental caries; and a quantitative model (stock and flow diagram) with the sets of mathematical equations. SSB tax policy implementation and 2 hypothetical scenarios were simulated to estimate the changes of dental caries prevalence of Thai children from 2017-2040.

Results: The prevalence of dental caries of Thai children (2-15 years) is projected to decrease in all simulation scenarios comparing with base-case. With the SSB tax only, dental caries prevalence is projected to reduce by only 0.48 % for primary teeth in urban area, and 2.82 % for permanent teeth in rural area by 2040. When combining other measures (reducing 20% sugar content in beverages, population level health promotion strategy), the prevalence of dental caries is projected to reduce further to 4.24% for primary teeth in urban area and 8.19 % for permanent teeth in rural area.

Conclusion: SSB tax alone is unlikely to impact on dental caries for Thai children unless it is accompanied with a comprehensive public health policy that aims to reduce total sugar consumption.

Keywords: Dental caries, Sugar-sweetened beverage tax, Sugar consumption, System dynamics model, Children

PA-00043

High Nitric Oxide Induces Autophagy in Head and Neck Cancer Cells

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Poster Presentation

ABSTRACT

Background and Objective: Autophagy is defined as "pro-survival functions" of the cells in response to stressful stimuli. Activation of autophagy has been reported in oxidative stimuli-induced cancer cells. However, autophagic response to stressful condition in head and neck squamous cell carcinoma (HNSCC) remains unclear. This study aimed to investigate the effect of high nitric oxide (HNO) on autophagic mechanism in HNSCC cell lines.

Methods: Isogenic primary (HN18 and HN30) and metastatic (HN17 and HN31) cell lines were used in the study. HNO-adapted cells were induced by nitric oxide donor (DEA-NONOate) for 72 h, and cell viability was determined using MTT assay. DEA-NONOate concentration which reduced cell viability < 10% was defined as HNO. The HNO-adapted cells were collected and cultured for autophagy testing. Early autophagic characteristic (acidic vesicular organelles) and apoptosis, were evaluated by flow cytometry.

Results: HNO concentrations for HN18, HN17, HN30 and HN31 cells adaptation were 3, 2, 4 and 4 mM, respectively. HNO-adapted HN30 cell showed significantly increased acidic vesicular organelles compared with its parent cell ($P < 0.05$). Whereas acidic vesicular organelles were significantly decreased in HNO-adapted HN18, HN17 and HN31 cells. Apoptosis in HNO-adapted HN18 cell was significantly greater than the parental group ($P < 0.05$). There was no apoptotic change in HNO-adapted HN17, HN30 and HN31 cells.

Conclusion: We demonstrated different HNO response between HNSCC cell lines. HNO-induced autophagy was observed by increasing acidic vesicular organelles in HNO-adapted HN30 cell. Additionally, apoptotic response to HNO was observed in HN18 cell.

Keywords: Autophagy, Apoptosis, High nitric oxide adaptation, HNSCC

PA-00044

Comparison Buccal versus Palatal Miniscrews of Maxillary Anterior Intrusion: A FE Study

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ABSTRACT

Background and Objective: To compare the pattern of initial tooth displacement and stress distribution between the palatal miniscrew-supported iPanda and conventional buccal anchorage systems using Finite element (FE) analysis.

Methods: The FE model of maxilla was constructed. Two different conditions of intrusion mechanics were simulated; in the iPanda condition, the miniscrews were placed on the midpalatal suture and an archwire were bonded to the maxillary first molars (iPanda). An intrusion arch was inserted into the bracket tubes on the maxillary first molars and applied the intrusive forces. In the buccal condition, the miniscrews were placed between the lateral incisors and canines and applied the intrusive forces to miniscrew. In each condition, under 60 gf of intrusive force, initial displacement and the von Mises stresses of the individual anterior and first molar teeth was measured.

Results: The anterior teeth underwent intrusion, accompanied by a labial tipping movement. The iPanda showed that central and lateral incisors presented the similar initial displacement pattern. On the contrary, the buccal miniscrew showed the most initial displacement at a lateral incisor. The maximum von Mises stress was observed at apical-palatal area in the PDL of anterior teeth in both conditions. Nevertheless, it was observed along mesial surface PDL of first molars with minimal displacement due to iPanda anchorage.

Conclusion: Intrusion with iPanda is an alternative treatment approach to effectively intrude maxillary anterior teeth without additional miniscrews. Moreover, this mechanic can reduce the risk of extrusion on maxillary first molars.

Keywords: Finite element analysis, Miniscrew, Intrusion

PA-00045

Diagnosis of Ankylosis in Permanent Incisors using AnyCheck Device

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Poster Presentation

ABSTRACT

Background and Objective: Ankylosis is a common complication of replanted or traumatized permanent incisors and results in the lack of physiologic mobility. Diagnosis of ankylosis can be made clinically by assessing the characteristic metallic sound emitted when the tooth is tapped. Therefore, the purposes of the present study were 1) to evaluate the possibility of performing the diagnosis of ankylosis using the damping capacity method with the AnyCheck device, and 2) to compare the tooth stability (TS) values between maxillary incisors with and without ankylosis.

Methods: The ankylosis group (n= 20) was composed of subjects who had at least one documented ankylosed tooth. The control group (non-ankylosis) (n= 20) was selected from a convenience sample of pre-orthodontic young subjects with healthy natural dentition free of pathologies (age range 15–19 years). Assessment of TS was performed using the AnyCheck device (AnyCheck, DMS Co., LTD. Gangwon-do, Korea). This device uses the tapping method which measures the time the tapping rod of the device contacts the tooth. The result of measurement is displayed in the 0-99 scale with a higher scale representing greater stability. An independent t-test was used to perform comparisons between groups.

Results: TS values from ankylosed maxillary incisors (83.5 ± 4.3) were significantly higher (approximately 30%) than the non-ankylosed baseline maxillary incisors (65.5 ± 7.1) group, $p<0.01$.

Conclusion: Assessment of damping capacity is an efficient diagnostic method to diagnose the presence of ankylosis before orthodontic treatment. Ankylosed incisors exhibited higher TS values than non-ankylosed incisors.

Keywords: Ankylosis, Damping capacity, Tooth stability, Orthodontic tooth movement

PA-00046

Orthodontic Management of Severely Traumatized Maxillary Incisors: 17 years Follow Up

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ABSTRACT

Background and Objective: Severe dental and maxillofacial injuries, which are associated with motorcycle accidents, have lifetime consequences. Even with a full coverage helmet, teeth often sustain major cracks, fractures, intrusion, and avulsion from the impact. A comprehensive assessment and timely treatment are especially important with dislodged or avulsed teeth. The adjunctive orthodontic treatment applied at the time of trauma combined with sensitive biomechanical options is essential for achieving satisfactory results. The purpose of this article is to present the long-term follow-up results of the comprehensive orthodontic management of severely traumatized maxillary incisors.

Methods: A 23-year-old female patient was referred for clinical evaluation following a motorcycle crash. The clinical assessment demonstrated the laceration of the lower lips and severe intrusion (12 mm) of all maxillary incisors with the oblique crown fracture of the right central incisor. No radicular fracture was observed on radiographic examination. Immediate traction of the incisors was planned with the initial 0.16-inch Sentalloy archwires. The patient was scheduled for a weekly follow-up for applying progressive orthodontic extrusion forces. After the clinical crown has been exposed, the patient was referred for endodontic evaluation and treatment. The orthodontic treatment for the extrusion of the maxillary incisors was completed in 3 months. Restorative treatment was performed to improve the patient's aesthetics. Excellent results well maintained after a 17-year follow-up revealed stable and pathologic free roots for all incisors.

Conclusion: The comprehensive orthodontic management of severely traumatized maxillary incisors and timely treatment is important for long-term life-changing results.

Keywords: Dental trauma, Orthodontic forces, Extrusion forces

PA-00047

Smart Springs for Orthodontic Extraction of Impacted Mandibular Third Molars

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Poster Presentation

ABSTRACT

Background and Objective: Impact mandibular third molars (iM8s) are difficult to retrieve and unsuitable to be used as donor teeth. Therefore, the study introduces the Smart Springs for controlled uprighting of iM8s to allow atraumatic extraction.

Methods: Sixty patients were enrolled and divided into three groups. In the MI8 group (n = 20), Smart Springs were anchored to miniscrews, while in the minimally invasive braces (MIB) group (n = 20), a reinforced dental anchorage was used to upright the iM8s. In the control group (n = 20) iM8s were removed by traditional methods. Panoramic radiographs were recorded at the beginning (T0) and by the end of the iM8 uprighting (T1). Treatment duration and total surgical time to extract the iM8s were recorded. Assessment of iM8 displacement and inclination was performed using custom-made software (Smart'n Ceph). A significance of 0.05 was used.

Results: All teeth were uprighted successfully. The application of orthodontic force was significantly shorter in the MI8 group (6 ± 2.5 weeks) than in the MIB group (8 ± 3.8 weeks) ($P<0.01$). No significant difference in the extraction duration between the MIB (1.4 ± 4.1 minutes) and MI8 (2.2 ± 2.1 minutes) groups was observed. No postoperative pain or discomfort was observed. In contrast, in the control group (28.4 ± 10.5 minutes) patients had experienced varying degrees of facial swelling and pain. The symptoms persisted for two to eight weeks.

Conclusion: Smart Springs anchored to either MI or MIB allow atraumatic extraction of iM8 with benefits for tooth autotransplantation.

Keywords: Orthodontic extraction, Impacted mandibular third molars, Tooth autotransplantation, Miniscrew implants, Dental anchorage

PA-00048

Tenting Graft Method for Alveolar Ridge Augmentation using Tooth Autotransplantation: Case Series

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ABSTRACT

Background and Objective: The periodontal ligament has the unique ability to perform osteoinduction that can be significantly enhanced by the application of orthodontic loading. The purpose of this study was to evaluate the effectiveness of using pre-loaded tooth autotransplantation (TAT) in a "tenting" fashion, to augment severely atrophic alveolar ridge defects. The new augmented ridges are used for both TAT and orthodontic tooth movement.

Methods: This prospective case study evaluated augmentation in five consecutive patients with severely resorbed alveolar ridges. Before augmentation, all alveolar sites were considered inadequate for TAT or orthodontic tooth movement. Horizontal ridge augmentation was performed using pre-loaded TAT to tent out the soft tissue matrix and periosteum for the adjacent particulate xenograft and CGF membrane. The ridges were evaluated using CBCT images at pre- and 3 months postoperative.

Results: Successful alveolar ridge augmentation with adequate horizontal width for TAT and orthodontic tooth movement was obtained. Augmented ridges became a recipient site for TAT and orthodontic tooth movement. Linear analysis of the width and volume of the recipient area at different time points revealed a final average increase in width was 5.9 ± 2.2 mm and a volume gain of 8.5 ± 3.2 mm^3. All augmented ridges had retained their functional and esthetic integrity at 1 year after original augmentation.

Conclusion: Alveolar ridge augmentation using pre-loaded TAT maintains space and minimizes resorption of the particulate xenograft volume. This innovative technic offers a predictable functional and aesthetic reconstruction of localized defects without extensive amounts of autogenous bone.

Keywords: Alveolar ridge augmentation, Tooth autotransplantation, Periodontal ligament, Osteoinduction, Orthodontic tooth movement

Poster Presentation

Poster Presentation

PA-00049

The Relationship between Professional and Normative Orthodontic Treatment Needs in Mae Chan Students, Chiang Rai

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ABSTRACT

Background and Objective: To study the relationship between the professional and normative orthodontic treatment needs among students between 13-15 years old in Mae Chan, Chiang Rai.

Methods: The study was a cross sectional analytic study. The samples included 13-15 years old students (n=327) from Mae Chan Wittayakhom school and Chan Chawa Witthayakhom school using the stratified random sampling. The samples were examined for professional orthodontic treatment needs using a DAI score which was evaluated by dental students. The normative orthodontic treatment needs were received from the questionnaire by samples. The investigators' reliability was calibrated by an orthodontist. The relationship were calculated by Fisher Exact Probability test and logistic regression test.

Results: According to the DAI results, 33.33% (n=109) of the participants presented indicating elective treatment, 25.10% (n=82) had no treatment need or slight need, 22.90% (n=75) had required highly desirable treatment and 18.7% (n=61) had mandatory treatment. Normative treatment needs showed that participants had barely need necessary treatment (61.08%) is greater than participants who don't need treatment (38.92%). The significant relationship between the professional treatment needs and normative treatment need (P value < 0.05). Increasing the professional need (DAI) level related to the increasing of the normative need.

Conclusion: The relationship between the professional and normative orthodontic treatment needs among students between 13-15 years old in Mae Chan, Chiang rai have highly significant relationships.

Keywords: Dental aesthetic index (DAI), Normative treatment need, orthodontics

PA-00050

Comparison of failure modes of two pit and fissure sealants using conventional etch and self-etching primer: An *In Vitro* study

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ABSTRACT

Background and Objective: Sealant using self-etch primer may be beneficial for prevention in uncooperative children because etching and rinsing steps could be skipped. Failure analysis could indicate the characteristics of sealant bonding after sealant dislodged. This study aimed to evaluate the failure modes of two sealants using different bonding systems.

Methods: Forty extracted premolars were randomly divided into 2 groups (N=20) including Group 1: Sealant using self-etch primer (BeautiSealant) and Group 2: Resin sealant combined with conventional bonding system (Scotchbond™Etchant gel combined with ClinproTMSealant). Sealant material was applied on flat enamel at the size of 2 mm diameter x 1 mm height. Specimen were subjected to thermocycle 500 cycles. Shear force was applied at the sealant-enamel interface and the failure mode of the specimens were examined under stereomicroscope. Failure modes were classified into four types.

Results: Failure mode analysis revealed that all specimens showed two out of four types, that are adhesive failure and mixed failure. There were no cohesive failure and enamel failure. Group 1 showed adhesive failure at 60% and mixed failure at 40%. Group 2 showed adhesive failure at 65% and mixed failure at 35%. There was no statistically significant difference in failure mode between group 1 and group 2 (Fisher's exact test, p=1.000).

Conclusion: After shear force test, percent of mix failure mode seen in both groups were comparable. Forty percent of the sealant using self-etch primer had mixed failure mode, suggesting that some resin remained on the surface after dislodged.

Keywords: Self-etching primer, Conventional etch, Resin sealant, Pit and fissure sealant, Failure mode

PA-00051

Third Molar Presence and Maxillary Posterior Bone Availability in Different Maxillary Types

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Poster Presentation

ABSTRACT

Background and Objective: Maxillary molar distalization is an important treatment alternative to extractions. The maintenance of the alveolar bone is tooth-dependent, therefore the presence of the third molar is important for the bone availability in the maxillary tuberosity area. The purpose of study was to compare the amount of bone availability in the presence and absence of the third molars among different maxillary types.

Methods: Sixty maxillary tuberosity sites were assessed using cone-beam computed tomography. The occlusal plane was defined by connecting the buccal cusp of the maxillary first premolar and both mesiobuccal cusps of the maxillary first molars. The distances between the mid-distal of distobuccal roots of the maxillary second molar and the inner cortex of the maxillary tuberosity were measured parallel to the occlusal line at depths 6, 8, and 10 mm apical to the cementoenamel junction of the second maxillary molar.

Results: In all depths, the posterior bone availability was significantly higher in the presence (8.2 ± 1.4 mm) than in the absence (4.6 ± 1.4 mm) of a third molar ($P<0.01$). In the absence of third molar, at all depths, the prognathic maxilla (5.4 ± 1.5 mm) had the greatest posterior bone availability, followed by the retrognathic (4.6 ± 1.9 mm) and orthognathic (3.9 ± 1.7 mm) maxilla. However, in the third molar group, no significant differences in posterior bone availability among different maxillary types.

Conclusion: The presence of the third molar plays an important role in posterior bone availability.

Keywords: Third molar, Maxillary molar distalization

PA-00052

The Efficacy of Handwashing Gel Containing Banana Peel Extract in Reducing Bacteria on Dentists' Hands

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ABSTRACT

Background and Objective: To support the utilization of banana peels as antibacterial agent, this study aimed to investigate the efficacy of handwashing gel containing banana peel extract in reducing bacterial load on dentists' hands.

Methods: Twenty-four volunteers were randomly distributed into 3 groups which subsequently underwent a rotation of washing hands with 4% chlorhexidine gluconate solution (CHG), gel with banana peel extract (test), and gel without banana peel extract (control). Before starting the trial, the volunteers washed their hands with 4% CHG, put on gloves, and provided dental treatments. After 30 minutes, they took off gloves and put thumbs on the first nutrient agar plates (baseline). The volunteers in each group then washed their hands with the assigned handwashing products and put thumbs on the second agar plates (immediately after washing the hands). They put on gloves and continued to do their work for 1 hour, then took off gloves and put thumbs on the third agar plates (one hour after washing the hands). After 24-hours incubation at 35°C, the bacterial colonies on agar plates were counted.

Results: The number of bacterial colonies was significantly decreased from baseline to immediately after washing the hands with 4% CHG and the test group but not the control group. (Wilcoxon signed-ranks test) However, the number of bacterial colonies was significantly increased from immediately to one hour after washing the hands for all studied handwashing products (Mann-Whitney U test).

Conclusion: Handwashing gel containing banana peel extract has the promising results in reducing bacterial load on dentist's hands.

Keywords: Bacterial load, Banana peel extract, Dentists' hands, Handwashing gel

PA-00053

The Efficacy of the Coloring Agents from Black Glutinous Rice on Dental Biofilm Staining

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Poster Presentation

ABSTRACT

Background and Objective: In search of an alternative dental disclosing solution with good staining ability and biocompatibility, this study aimed to investigate the efficacy of the coloring agents from black glutinous rice on dental biofilm staining and compare to erythrosine dye.

Methods: Black glutinous rice (BGR) was boiled and frozen dry to get the dark purple powder, which was later mixed with water at ratio 1:2 before application. Thirty-six volunteers were classified into 3 groups (n=12) according to their age (18-26, 27-46, and 47-66 years old). The crossover clinical trial was conducted by applying either erythrosine dye (ED) or BGR solution. The disclosing agents were applied on the tooth with cotton swabs, the examiner and the volunteers reported the stained tooth, as yes or no, for 4 sites/teeth. After 2-weeks washout period, the volunteers switched to apply another agent.

Results: Kappa coefficient demonstrated the interrater reliability between the examiner and the volunteers of ED and BGR solution at 0.338 and 0.295 respectively. It illustrated the same interval of agreement of both disclosing agents**.** There was no statistically significant difference among different aged groups. The analysis of tooth location and tooth surfaces showed the significant difference in lower posterior teeth and mesial surface, respectively (Paired t-test; $p<0.05$).

Conclusion: The coloring agents from black glutinous rice can be used for dental biofilm staining, however, its efficacy is slightly less than erythrosine dye. It shows the potential to be used as an alternative disclosing agent which is natural, safe and convenient for household using to promote oral hygiene care.

Keywords: Black glutinous rice, Dental biofilm staining, Disclosing agent, Erythrosine

PA-00054

The Efficacy of Mouthwash Containing Essential Oil from *Zanthoxylum limonella* Alston on Dental Biofilm and Gingivitis Controls

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ABSTRACT

Background and Objective: Ma-Khwaen, locally Thai word of *Zanthoxylum limonella* Alston, is generally grown in the North of Thailand. The aim of this study was to evaluate the efficacy of mouthwash containing essential oil from the fruits of Ma-Khwaen on dental biofilm formation and gingival inflammation.

Methods: Twenty-four volunteers were participated in this randomized crossover clinical trial. After receiving professional prophylaxis and waited for 14 days, the volunteers were allocated into three groups: 0.12% chlorhexidine digluconate (CHX); 0.5% Ma-Khwaen mouthwash (Ma-Kh); distilled water (DW), and started to rinse with 20 ml of their respective mouthwashes for 1 minute, immediately after brushing in the morning and at night for 14 days. Clinical parameters PI and GI were conducted on baseline, Day 7 and Day 14. At the end of each experimental round, the volunteers underwent 2 weeks rest period before starting the second and third allocations.

Results: The results showed that both CHX and Ma-Kh groups demonstrated the reduction on PI and GI statistically significant difference from baseline to Day 7 and Day 14 (Wilcoxon Signed Ranks test $P < 0.05$). There was also statistically significant difference when compared the efficacy in PI and GI reduction between CHX and DW groups as well as Ma-Kh and DW groups, but no significant difference between CHX and Ma-Kh groups (Mann-Whitney U test $P < 0.05$).

Conclusion: This study shows that Ma-Khwaen mouthwash demonstrated clinical efficacy on dental biofilm and gingival inflammation reduction. It seems to be possible an alternative mouthwash for gingivitis control.

Keywords: Dental biofilm, Essential oil, Gingivitis, Mouthwash, *Zanthoxylum limonella* Alston

PA-00055

Okra Jelly Affecting Self-perceived Xerostomia and Oral Health-related Quality of Life in the Elderly: A Preliminary Study

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Poster Presentation

ABSTRACT

Background and Objective: The prevalence of xerostomia is quite high in elder people. Okra contains mucilaginous substance which has moisturizing and lubricating properties similar to human natural saliva. This study aimed to investigate if okra jelly can affect self-perceived xerostomia and oral health related quality of life in the elderly.

Methods: Twelve participants were randomly allocated into 2 groups; to have okra jelly or jelly without okra. They received jelly twice daily, between breakfast-lunch and lunch-dinner for 24 days. Self-reported visual analogue scale (VAS) and the Thai version of Oral Health Impact Profile-14 (OHIP-14-Th) had been done at before intervention, Day 12, and Day 24. Two-sample Wilcoxon rank-sum (Mann-Whitney) test, multilevel mixed-effects logistic regression and GEE population-averaged model were used for analyzing the differences between tests and controls at different studied times with p-value < 0.05 was considered to be significant.

Results: After adjusting baseline VAS and OHIP-14-Th score, age and gender, the results showed that every 12-day, the VAS score of the test group decreased significantly ($p < 0.01$) and the control group decreased insignificantly. Comparing the two groups, it was found that the test group had a greater score reduction significantly ($p < 0.01$). The OHIP-14-Th score every 12-day showed that both the test and control groups decreased significantly ($p < 0.01$). Comparing the two groups, it was found that the test group had a greater score reduction, however, insignificantly.

Conclusion: Okra jelly seems to have promising results on the reduction of self-perceived xerostomia and oral health related quality of life in the elderly.

Keywords: Elderly, Okra, Quality of life, Xerostomia

Extended Abstracts for Competition

(Undergraduate)

10th Anniversary
SCHOOL OF DENTISTRY
Mae Fah Luang University
DFCT2022
Chiang Rai, Thailand

undergraduate OC-00007

Effectiveness of Iodine for Continuous Decontamination of Dental Unit Waterline

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EXTENDED ABSTRACT

Introduction: Dental Unit Waterlines (DUWLs) are prone to colonization by biofilms that release various species of microorganisms into dental unit output water. Controlling DUWLs contamination is therefore crucial in infection control in dental practice. This study aimed to investigate the effectiveness of devices continuously releasing Iodine to control microbial contamination in DUWLs.

Materials and Methods: Ten dental chair units (DCU) at Chulalongkorn university were randomized into the iodine and control groups. After installing Iodine cartridge, the DCUs were let to operate normally. The DCUs output water was collected from airotors lines for enumerating bacterial contamination weekly for nine weeks. At the end of the experiment, the viability of the biofilm in the DUWLs was indirectly quantified by the ATP testing kit. The amount of Iodine released in output water was also measured by the Iodide electrode.

Figure 1: Iodine cartridge water treatment as an adapter cartridge can be easily installed with an independent water reservoir bottle of the dental unit. It does not need an electrical supply, is inexpensive, and is easy to install.

Results and Discussion: The continuous presence of Iodine in the DUWLs could significantly control bacterial contamination to meet the CDC standard level (less than 500 CFU/mL). The Iodine treatment can reduce bacterial CFU in the DUWLs up to 98 to 100 percent. The number of bacteria was drastically decreased in the Iodine group from the first week of treatment.

Biofilm viability demonstrated a trend decrease in the Iodine group though not statistically significant. An average iodine concentration of 3.66 ppm was detected in DCUs output water in the Iodine group but not in the control group. We found that continuous

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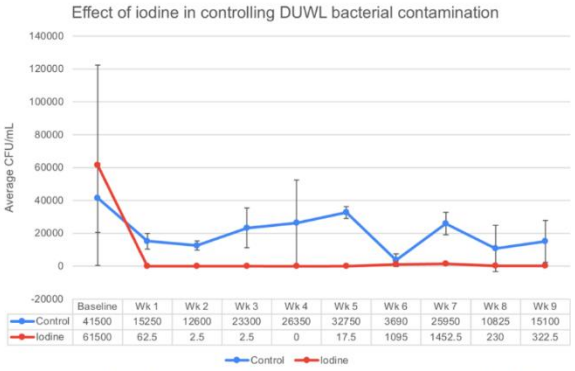
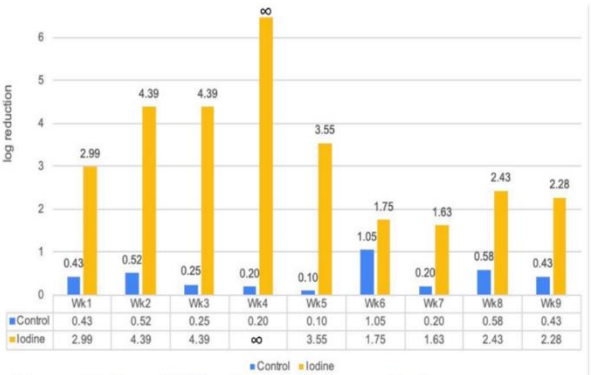


	Baseline	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9
Control	41500	15250	12600	23300	26350	32750	3690	25950	10825	15100
Iodine	61500	62.5	2.5	2.5	0	17.5	1095	1452.5	230	322.5

Figure 2: The effect of Iodine in controlling DUWLs bacterial contamination. The average number of bacterial counts (CFU/mL) is presented in the table below the chart.



	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8	Wk9
Control	0.43	0.52	0.25	0.20	0.10	1.05	0.20	0.58	0.43
Iodine	2.99	4.39	4.39	∞	3.55	1.75	1.63	2.43	2.28

Figure 3: Log CFU reduction between Iodine group and control group.

iodine treatment effectively controlled bacterial contamination in DUWLs from the beginning after installation. However, it is less likely to deprive biofilms viability as measured by the amount of ATP.

Several continuous treatments have been proven as effective methods to decontaminate DUWLs, such as plasma sterilization, Ozonated water, electrolyte water system, or hypochlorous acids. These systems usually need the automated machine to freshly prepare the disinfecting agent in real-time at the point of use. Besides providing disinfecting agents continuously, the automated systems are effective because no staff compliance need; on the other hand, they can reduce human error. However, the automated system is costly and requires prompt maintenance. Meanwhile, the Iodine cartridge water treatment is commercially available as an adapter cartridge that can be easily installed with an independent water reservoir bottle of the dental unit. It does not need an electrical supply, is inexpensive, and is easy to install. Thus, it needs less attention than the automated system. The longevity of Iodine released from the cartridge is limited to the amount of water passed through the cartridge at approximately 240 liters. Thus, it is necessary to track water input. Some people should be concerned about the toxicity of iodinated water to vulnerable patient groups such as Iodine allergies, including seafood, pregnancy, lactation, and some medical condition such as autoimmune thyroid disease or person with chronic iodine deficiency. In case of excessive iodine intake in vulnerable patient groups, it can cause alterations in thyroid function. Therefore, taking a medical history is essential to prevent adverse events. It is needed to inform recommendations about Iodine safety doses for vulnerable patient groups.

Conclusion: Continuously supplying Iodine in DUWLs is an effective measure to control microbial contamination in DUWLs. Therefore, this study recommended Iodine as an alternative treatment for decontaminating the dental unit waterlines.

Keywords: Dental Unit Waterlines, Continuous treatment, Iodine treatment, Biofilm, Water contamination

undergraduate OC-00008

Effects of Multiple Firing on the Color and Translucency of CAD-CAM Glass-Ceramic

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EXTENDED ABSTRACT

Introduction: Ceramic restoration has become more popular than other dental restorative materials because of its superior esthetics and physical properties. Furthermore, the colour and translucency are key factors that influence the aesthetic of the restoration, such as crowns, bridges or veneer. By using computer aided designs and manufacturing (CAD/CAM) systems, the ceramic block is passed through a manufacturing process, which contains staining, glazing and firing process. Sometimes the fabrication process may require multiple firing for colour correction, until the dentist or the patient get their desired final restoration. More importantly, the multiple firings may affect the colour and translucency of the ceramic. Therefore, our research question is 'Does multiple firing affect the colour stability and translucency of cad-cam glass ceramic of each brand differently?' The research hypothesis is that multiple firings significantly affects the colour stability and translucency of cad-cam glass ceramic of each brand in a different way. The purpose of this research is to study the effect on Lithium Disilicate (LDS) and Zirconia Reinforced Lithium Silicate (ZLS) after multiple firings in order to perform colour correction accurately before delivering the restoration.

Materials and Methods: Blocks of LDS (IPS™ e.max CAD and HASS Amber Mill) and ZLS (Celtra®Duo and Vita Suprinity) were selected for this study, twelve specimens from each trademark. A total of 48 specimens were cut perpendicularly using a low-speed diamond saw into 1± 0.5 x 14 x 20 mm^3 shapes. The firing was performed 3 times and on completion of each firing, the specimens were measured for colour and translucency. The values of contrast ratio (CR), translucency parameter (TP) and colour difference (ΔE) were recorded and evaluated using repeated-measure Anova in order to compare the values.

Results and Discussion: When comparing trademark brand, HASS Amber Mill exhibited the highest translucency parameter (TP) and lowest contrast ratio (CR) in every firing cycle. When comparing each trademark during different firing, Vita Suprinity showed significantly lower in TP in the second firing and HASS Amber Mill showed significantly lower in TP in the third firing. However, no significant difference was found in TP of Celtra ®Duo and IPS™ e.max CAD after the multiple firings. The CR in the second and third firings was increased in HASS Amber Mill. Nevertheless, other trademarks (Celtra®Duo, IPS™ e.max CAD and Vita Suprinity) had no significant difference in CR. In the first and second firings, Celtra®Duo and Vita Suprinity

Competition

illustrated the highest CR compared with other brands. However, in the third firing, Celtra®Duo showed the highest CR. Additionally, the colour difference (ΔE), in the second firing Vita Suprinity and IPS™ e.max CAD are between 0-1, Celtra®Duo and HASS Amber Mill are between 2-3.5. After third firing the colour difference of IPS™ e.max CAD is between 1–2, Celtra®Duo and HASS Amber Mill are between 2–3.5, Vita Suprinity was more than 5.

Comparing results with previous studies, the corresponding outcome was no significant difference found in TP of IPS™ e.max CAD after third firing. Therefore, our first aim is to expand the existing body of knowledge of the previous studies by conducting the experiment of dental ceramics more recently used in Thailand, such as Celtra®Duo, IPS™ e.max CAD, Vita Suprinity and HASS Amber Mill. Besides the translucency parameter and contrast ratio, the color difference is another important point that our team added to the study with the intent of evaluating divergent factors with no current data.

Conclusion: The multiple firing process affects the colour stability and translucency of ceramic restoration. For the colour stability, HASS Amber Mill and Celtra®Duo have a remarkable change after the second firing. Furthermore, after the third firing, IPS™ e.max CAD illustrated a minor change in colour. However, after the third firing, Vita suprinity exhibited the highest value of colour change in our experiments compared with other brands. For the translucency, there was a significant decrease in translucency in the second firing of Vita Suprinity and in the third firing of HASS Amber Mill, while Celtra®Duo and IPS™ e.max CAD showed no significant difference of translucency during firing process. However, HASS Amber Mill represents the highest translucency value in comparison with other brands in every firing cycle.

Keywords: Color difference of ceramic, Contrast Ratio ceramic, Multiple firing ceramic, Repeat firing ceramic, Translucency Parameter ceramic

undergraduate OC-00010

Melatonin Prevents H_2O_2-Induced Premature Senescence in Human Gingival Fibroblasts

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EXTENDED ABSTRACT

Introduction: Periodontitis is described as a disease of oxidative stress where excessive reactive oxygen species (ROS) are generated by invading bacteria and host immune response resulting in the destruction of the cells in the periodontium, including the prominent resident cells, the gingival fibroblasts. Gingival fibroblasts play many important roles in the homeostasis of the periodontal health, including matrix turnover, hard tissue remodeling, and modulation of inflammation. Therefore, oxidative damage to the fibroblasts will result in the disruption in these processes.

Cellular senescence, or aging of cells, is the process when cells undergo cell cycle arrest, change their morphology, resist apoptosis and alter their gene expressions. Exposure to excessive ROS or strong chemical agents can lead to premature cellular senescence or stress-induced senescence. Since the senescent fibroblasts have been shown to contribute to the progression of periodontitis, preventing senescence can thus decrease severity of the disease.

Melatonin is a neurohormone and well-known for its role in the regulation of circadian rhythm, but most importantly for its antioxidant properties. While several studies have shown a role of melatonin in modulating periodontitis, whether melatonin can prevent senescence in gingival fibroblasts exposed to oxidative stress requires further investigation.

Therefore, our study was performed to investigate the effect of melatonin on H_2O_2-induced senescence in human gingival fibroblasts.

Materials and Methods: **Phase 1**: This phase was performed to determine the optimal concentration of H_2O_2 for induction of senescence. Human gingival fibroblasts (HGFs) were treated with H_2O_2 at different concentrations (40 - 80 µM) for 5 days as the experimental groups or cultured in complete medium as the negative control. Medium or medium with H_2O_2 was changed every day. Subsequently, HGFs were subjected to senescence-associated β-galactosidase (SA-β gal) staining which is commonly used as a marker for cellular senescence. Cell morphology was investigated using an inverted light microscope and reported by description. Cells were photographed for analysis of the SA-β gal positive cells. The numbers of SA-β gal positive and negative cells were counted in a total of at least 200 cells from 3 different fields and the percentages of positive cells were calculated using the number of positive cells divided by total counted cells. The number of positive cells at each different concentration should be at least 50% to be considered as an optimal concentration to induce senescence.

Competition

Phase 2: This phase was performed to study whether melatonin could prevent senescence of the gingival fibroblasts. HGFs were treated with melatonin at 10 nM, 100 nM, 1 µM, 10 µM and 100 µM or cultured in complete medium with 0.05% dimethyl sulfoxide (DMSO) as the negative control for 2 hours. Then, cells were washed twice with phosphate-buffered saline (PBS) before being treated with the optimal concentration of H_2O_2. Melatonin alternating with H_2O_2 treatment was performed every day for 5 days. Subsequently, HGFs were subjected to SA-β gal staining and cell cycle analysis using flow cytometry. The percentages of the SA-β gal positive cells and cells in G0/G1 or G2/M phases of the groups treated with melatonin were compared to the negative control and the group treated with H_2O_2 alone.

Statistical analysis: Each experiment was done in duplicate and repeated at least 3 times independently. One-way analysis of variance (one-way ANOVA) with Tukey's test was calculated for comparing the percentages of SA-β gal positive cells and percentages of cells in each phase of cell cycle among experimental groups. A p-value < 0.05 was considered as statistical significance.

Results and Discussion: **Phase I**: H2O2 at 70 µM could significantly increase the number of SA-β gal positive HGFs to more than 50% of total cells while the negative cells remained normal. At higher H_2O_2 concentrations, the negative stained cells showed thin or thread like morphology which did not indicate the morphology of normal or senescent cells. Therefore, 70 µM H_2O_2 was then chosen as the optimal concentration to induce senescence.

Phase II: Although melatonin at 100 nM, 1 µM and 10 µM could significantly decrease the percentages of positive cells induced by H_2O_2, treatment with 1 µM melatonin resulted in lowest percentage of positive cells. Subsequently, the effects of 70 µM H_2O_2 and 1 µM melatonin were confirmed by cell cycle analysis. The result showed that H2O2 decreased the amount of cells in G0/G1 phase and increased the amount of cells in G2/M significantly, while melatonin treatment could attenuate these effects of H_2O_2 to the levels that were not significant from the control.

Taken together, our study suggested that melatonin could attenuate the process of senescence of HGFs induced by H_2O_2 as demonstrated by SA-β gal staining and cell cycle analysis. Although the cycle of senescent cells is usually halted at G1 phase,[1] substantial studies have shown that long duration of H_2O_2 treatment could also induce cell arrest at G2/M phase[2] which also suggested a characteristic of senescence. Consistent with our results, melatonin was reported to decrease SA-β gal activity of the ethanol-induced premature senescence of periodontal ligament cells.[3] The possible mechanisms include direct free radical scavenging and indirect pathway through melatonin receptors.[4] However, further studies are required to elucidate the mechanism of melatonin in mitigating senescence of HGFs.

With further in vivo experiments, melatonin can be a promising candidate to be used alongside with other conventional treatments in the management of periodontal disease such as an irrigation solution. In addition, because melatonin has been included in several daily life products such as milk or gummies for adjusting circadian rhythm with minimal side effects, it is likely that this hormone can also be included as an active ingredient in oral prophylactic products such as toothpaste or mouthwash to prevent cellular senescence of periodontium.

Conclusion: Melatonin treatment could decrease the number of SA-β gal positive cells induced by H_2O_2 and mitigate cell cycle arrest at G2/M phase. Our findings suggest that melatonin can be a potential substance to protect periodontium from oxidative stress that leads to cellular senescence.

Keywords: Cellular senescence, Human gingival fibroblast, Melatonin, Periodontitis

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undergraduate OC-00011

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Withdrawn

undergraduate OC-00014

Finite Element Analysis of KKU Smart Interlocking Sleep Device for OSA Therapy

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Competition

EXTENDED ABSTRACT

Introduction: Obstructive sleep apnea (OSA) is a sleep-related breathing disorder characterized by repeated episodic collapses of the upper airway during sleep, resulting in sleep deprivation. In Thailand, the prevalence of OSA is 15.4 % in male and 6.3 % in female. Treatment modalities that require patient's compliance are continuous positive airway pressure (CPAP) and mandibular advancement device (MAD). MAD is an alternative treatment for patients with mild-to-moderate OSA that are intolerable and poor compliance to CPAP. Since MAD had been increasingly prescribed for both OSA and primary snoring patients, however; the commercially available MADs are high-cost and unaffordable for some patients. Moreover, MAD has not been covered by Thai Universal Health Coverage Scheme. This study aimed to design and develop MAD for clinician and patient preferences. Furthermore, we also assessed strength and stability of the novel designed MAD and tested biomedical side effects on teeth, alveolar bone and temporomandibular joint using finite element analysis.

Materials and Methods: We performed surveys to identify clinician and patient needs. Three-dimensional skull model was constructed according to dental CBCT. The relationship between maxillary and mandibular teeth was registered at 50% of maximum protrusion and captured by TRIOS4 intraoral scanner. The skull model and newly designed were constructed by SolidWorks (Figure 1C) and polymethyl methacrylate (PMMA) was a material of choice. Stress distribution and displacement of newly designed MAD were analyzed by finite element analyses (FEA) under three simulated situations, including mandibular retraction, mandibular retraction with clenching, and mandibular retraction with lateral teeth grinding at half maximum (50%) and maximum protrusion, using ANSYS V20R2. Biomechanical side effects on teeth, alveolar bone and temporomandibular joint were also tested using FEA.

Results and Discussion: Novel designed MAD (Figure 1A) was constructed according to the need assessment in both dentist and patient groups. It comprised body and attachment apparatus components that worked together to maintain position of the mandible during sleep. This device included three components, maxillary splint, mandibular splint with lateral fin and protrusive button: a set of trapezoidal buttons which protrude the mandible by 0 to 7 mm gradually from 50% of maximum mandibular protrusion.

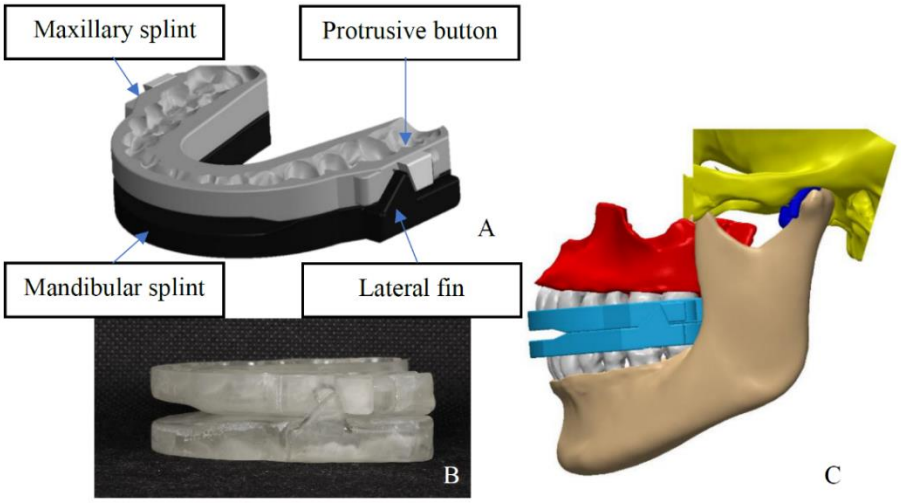

Figure 1. KKU Smart Interlocking Sleep Device in 3D model (A), 3D printed model (B) and insertion on skull model (C)

FEA showed that there were both compressive and shear stress affecting MAD. However, compressive stress was mainly acted on the device. Chromatic distribution (Figure 2) showed the maximum von Mises equivalent stress appeared at lateral fins of mandibular splint and the border of protrusive buttons. Nevertheless, the maximum compressive stress was not exceeded the compressive strength of PMMA in all situations. To reinforce the protrusive button, we re-designed by adding posterior supporter to share the compressive load.

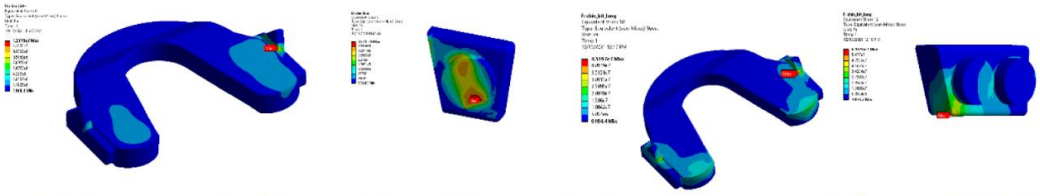

Figure 2. Chromatic distribution of von Mises equivalent stress on novel designed MAD

Biomechanical side effects of novel designed MAD showed uniformly distribution of compressive load on teeth and alveolar bone due to complete-coverage design. While, the forced protrusion caused by MAD did not cause considerably larger compressive loads on temporomandibular joint.

There is increasing implementation of digital dental technologies to facilitate dentists and patients. The digital workflow of processing the MAD could be done in one-visit by taking the intraoral scan and bite registration, custom designing by pre-formed MAD in the software and printing by 3D printer (Figure 1B). The advantages are higher accuracy and less chair time and laboratory time, compared to conventional technique. On the contrary, there is still higher initial cost of the hardware and software. In addition, the user needs to develop digital skills to proceed the treatment workflow.

Conclusion: Given our MAD was designed from the clinicians' and patients' demands, we believe that it would be practical for both of them. It has proper biomechanical properties withstanding clenching and grinding forces. Moreover, our biomechanical analysis of the effect of MAD on teeth, alveolar bone and temporomandibular joint exhibited no considerably increased stress in all tested simulations. Nevertheless, the clinical trial to evaluate the efficacy and side effects of MAD in patients are essential for the future study.

This KKU Smart Interlocking Sleep Device is under petty patent pending No. 2103003145.

Keywords: Mandibular advancement device, Obstructive sleep apnea, Finite element analyses, Biomechanical properties, Biomechanical side effects

undergraduate OC-00015

Effect of cavity conditioners on microleakage of zirconia reinforced glass ionomer in class V restoration

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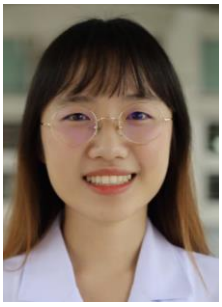

EXTENDED ABSTRACT

Introduction: Zirconia reinforced glass ionomer is a new glass ionomer restorative material that incorporated with nano zirconia fillers to improve material strength while remains its ability to release fluoride1. This material is introduced as the alternative restorative material for geriatric patients with class V cavities. However, microleakage was a major concern for this restoration. It has been reported that surface conditioning would increase the bonding strength between the glass ionomer restoration and tooth surface2. However, a concern regarding the improvement of microleakage after the use of different dentine conditioners prior to class V restoration with Zirconia reinforced glass Ionomer has remained. Therefore, this study aimed to evaluate the microleakage in class V Zirconia reinforced glass Ionomer restoration in cavities that were conditioned either with polyacrylic acid or ethylenediaminetetraacetic acid. A hypothesis of this study was the use of dentin conditioners would affect the microleakage of the restoration.

Materials and Methods: This research obtained ethical approval from the Ethics committees, Faculty of Dentistry, Chiangmai University (License no. 29/2564). Thirty intact third permanent molars were collected and kept in 0.5% thymol solution. Standardized class V cavities (2 mm x 3 mm x 2mm), located at 1 mm above the CEJ, were prepared either at the buccal or lingual surface. The samples were randomly divided into 3 experimental groups according to different cavity conditioner applications. *Group1*(control): no cavity conditioner, *Group 2*: 20% polyacrylic acid, and *Group 3*: 17% ethylenediaminetetraacetic acid. The conditioners were applied according to the manufacturer's instructions, then restored with zirconia reinforced glass ionomer. The samples were coated with petroleum jelly, and placed in distilled water for 24 hours at 37°C. Then the samples were thermocycled for 500 cycles in a water bath between 5 and 55°C with 30 seconds dwell time in each bath and 15 seconds for transferring time. All the teeth apices were sealed using pink wax. Two coats of nail varnish were applied to the external surfaces of all teeth leaving a 1 mm margin around the restorations. The samples were then immersed in 0.5% methylene blue for 24 hr at 37°C. Each tooth was divided buccolingually into a mesial and distal section. The microleakage was

Competition

analyzed under stereomicroscope at 20X magnification at 2 locations (occlusal and gingival margins of the restoration). The distance of dye penetration was measured using the ImageJ software. The mean percentage of dye penetration of each group was calculated and analyzed using a two-way ANOVA statistic (SPSS software).

Results and Discussion: The greatest mean percentage of dye penetration on occlusal margin was observed in the 20% polyacrylic group, followed by no cavity conditioner and 17% ethylenediaminetetraacetic acid group respectively. On the other hand, for gingival margin, the greatest mean percentage of dye penetration was observed in specimens without cavity conditioner, followed by 17% ethylenediaminetetraacetic acid and 20% polyacrylic group respectively. However, no significant differences were observed among the groups. These findings were correlated with a study by Kumari et al. 2022 that revealed no differences in microleakage of the restoration3. Focusing on the microleakage observed at the occlusal margin, zirconia reinforced glass ionomer showed less amount of microleakage, regardless of cavity conditioners used, when compared to the leakage at the gingival margin. One of the reasons could be the high mineral content in the enamel that might create the strong adhesion of the glass ionomer cement to the enamel surface4. For the microleakage at the gingival margin, the specimens with cavity conditioners, especially with polyacrylic acid, showed the least microleakage. The reasons that supported this finding would be the chemical reaction of the agent to the dentin collagen which might enhance the hydrogen bridging to peptide bond2. Therefore, the adhesion of the dentin is improved.

Conclusion: In this experiment setting, the use of cavity conditioners prior to restoring the class V cavity with zirconia reinforced glass ionomer did not affect the microleakage of the restoration. The application of either 20% polyacrylic acid or 17% ethylenediaminetetraacetic acid at the gingival margin tended to reduce the microleakage.

Keywords: Zirconia reinforced glass ionomer, Dentine conditioners, Microleakage, Class V

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undergraduate OC-00016

Development of Alveolar Ridge Model for the Practice in Mandibular Torectomy

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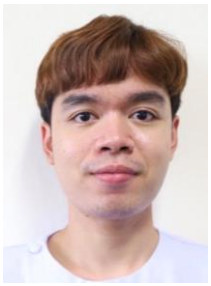

EXTENDED ABSTRACT

Introduction: Natural rubber gingival pad for suturing practice has been developed for dental students in Prince of Songkla University for a few years following the development of mechanical properties (tensile strength and shore hardness) of the natural rubber pad similar to the pig's attached gingiva[1] and the simulated jaw bone developed by using various combination ratios of fast-cured acrylic resin and type III dental gypsum which provided the physical properties as the porcine mandibular bone was previously reported by electric current measuring of the force when using bur drilling.[2] Mandibular torectomy is one of the usual surgical procedures for pre-prosthetic surgery. Nowaday, there is no simulated model for the starter oral surgeon to practice in torectomy procedures. Therefore, the challenging study aimed to fabricate the lower alveolar ridge model with simulated both right and left torus mandibularis which have bone hardness and gingival thickness similar to the natural bone and gingiva; and to assess the satisfaction of model practicing.

Materials and Methods:

1. The Vickers hardness test was measured by a microhardness tester (Mitutoyo, HM-211, Mitutoyo Corporation, Kanagawa, Japan) in four groups of mixture ratio of fast-cured acrylic resin and type III dental gypsum (1:1, 1:0.75, 1:0.5, 1:0.25) and one group of the pig jawbone.

2. The liquid natural rubber compound with added plasticizer was prepared for dipping. The gingival coverage was fabricated using regular dipping and dipping with a coagulation agent (5% w/v $CaCl_2$) in six cycles. The gingival thickness was measured by a measuring microscope (Nikon, MM-400, Nikon Corporation, Tokyo, Japan) and a comparison was performed between the two groups. The more thickness gained method was to be applied for model fabrication.

3. The alveolar ridge model preparation was obtained by constructing a silicone mold of the lower alveolar ridge with tori, then the suitable ratio of the bone materials and dipping method was used to fabricate the model. Then the model was dipped in the compound rubber to construct the gingival covering.

4. Satisfaction assessment on the simulated model was performed by 10 postgraduate students who had the experience of at least one case of torectomy and 30 cases of surgical removal of impacted teeth. The procedures to be assessed were soft tissue or flap procedure, bone removal, and suturing procedures. The satisfaction score ranged from 0-10 on the visual analog scale which 0 being “not satisfied at all” and 10 being “the most satisfied.” The items of the satisfaction questionnaire were pre-evaluated by the Item-Objective Congruence (IOC) based on a score range from -1 to +1. The items that had scores higher than or equal to 0.5 were reserved.

5. The data were analyzed using statistical software (SPSS Statistic Version 22). One-way ANOVA was performed to analyze differences in Vickers hardness among the 4 groups of acrylic resin mixed with type III dental gypsum and one mandibular bone group, and in gingival thickness of the two dipping methods. The satisfaction score was analyzed in descriptive.

Results and Discussion: The Vickers hardness of the four groups of mixture ratio of fast-cured acrylic resin and type III dental gypsum, ratios of 1:1, 1:0.75, 1:0.5 and 1:0.25, were 16.21, 17.31, 16.15, and 20.43 kg/mm^2 respectively. All four groups’ hardness was significantly lower than pig cortical bone (33.29 kg/mm^2) with p= 0.000. In the previous study of model drilling, the drilling force was similar to the drilling in the bone models because the cutting through both cortical and trabecular bone contributed to the realistic sensation. The Vickers hardness in this study was assessed on the cortical surface, therefore, resulting in a higher value than the simulated bone materials (the mixture of fast-cured acrylic resin and type III dental gypsum). However, the selected proportion of bone materials used for the model fabrication was determined by an oral surgeon who tried drilling, and the ratio of 1:1 was used to construct the simulated mandibular models.

The thickness of the coagulant dipping group (0.838 mm) was found to gain more thickness than the normal dipping method (0.220 mm). In the model dipping process, it was found liquid rubber could not flow to the surface of the narrow fold area. The syringe-injection technique was then applied to ease up the dipping in the narrow vestibule area at the first dipping process.

The overall satisfaction scoring was at a high level (score 7-10) in one hundred percent of the participants.

Conclusion: The alveolar ridge model for torectomy fabricated by using the mixture ratio of 1:1 and coagulant dipping can well simulate the sensation in flap operation, bone cutting, and suturing. Furthermore, it is more conveniently provided than fresh animal models.

Keywords: Natural rubber, Simulated model, Suturing, Torectomy

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Extended Abstracts for Competition

(Postgraduate)

10th Anniversary
SCHOOL OF DENTISTRY
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postgraduate OC-00001

Remineralization of Calcium Silicate Cement in Artificial Caries Affected Dentine

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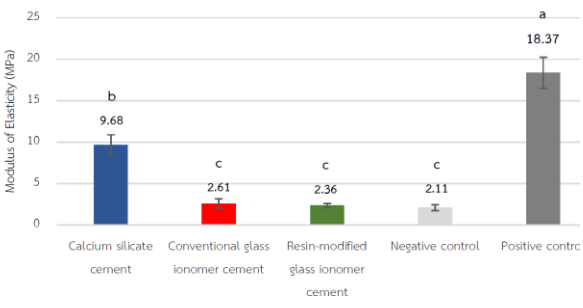
EXTENDED ABSTRACT

Background and Objective: Glass ionomer cement is widely used for dental caries treatment due to its remineralization, fluoride release, caries inhibition, and chemical bonding with dentin.1 Biodentine is a calcium silicate cement which first used in endodontic treatment. Several studies have discovered that in the calcium silicate group can stimulate remineralization.2 The objective of this study is to evaluate the efficacy of remineralization in artificially caries-affected dentine restored with three different materials: calcium silicate cement, conventional glass ionomer cement and resin modified glass ionomer cement.

Materials and Methods: 25 human third molars were prepared for Class V cavities on the buccal side, and 20 cavities were used to simulate caries-affected dentin using the pH cycling method. Then 15 cavities were filled with three groups of different materials: calcium silicate cement (n=5), conventional glass ionomer cement (n=5) and resin modified glass ionomer cement (n=5) and immersed in artificial saliva for one week. The five demineralized cavities without restoration served as the negative control group (n=5), while the five sound cavities served as the positive control group (n=5). All of the specimens were cut longitudinally, and a nanoindentation test was performed on the dentine beneath the material at the axial wall and two specimens of each group were measured using energy dispersive X -ray spectroscopy (EDX) and scanning electron microscope (SEM).

Results: The positive control group had the highest modulus of elasticity. The calcium silicate cement group was the second, followed by the conventional glass ionomer cement group, the resin-modified glass ionomer cement group, and the negative control group, respectively. The negative control group, the conventional glass ionomer cement group, and the resin-modified glass ionomer cement group were not statistically different. However, the elastic modulus of the calcium silicate cement group was higher than the other three restorative materials ($p<0.05$).

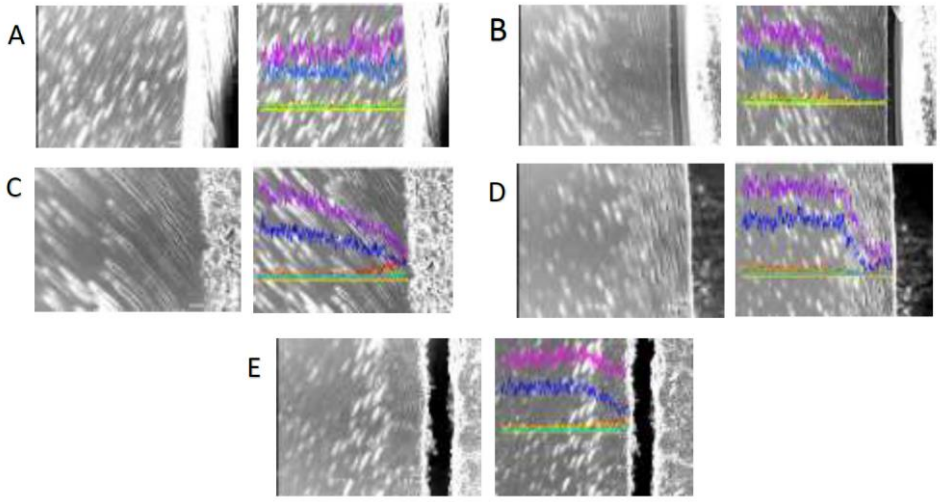


Mean Value of Modulus of Elasticity for 5 Groups
Groups labeled with the same letters do not differ significantly ($p > 0.05$

Competition

The EDX analysis showed higher calcium and phosphorus in the positive control group and calcium silicate cement group than in the conventional glass ionomer cement group, resin modified glass ionomer group and the negative control group.

The SEM analysis showed mineral deposits in the dentin layer of the calcium silicate cement group and the positive control group. However, there were no evidence of mineral deposits in the other three restorative materials.



A. Positive control group B. Negative control group C. Conventional glass ionomer cement group D. Resin modified glass ionomer cement group E. Calcium silicate cement group *Left: SEM image750x; Right: EDX line scan (Purple line = phosphorus, Blue line =calcium)

Glass ionomer cement and calcium silicate cement have different material compositions and properties; glass ionomer cement is acidic and undergoes remineralization via ion exchange, whereas calcium silicate cement has alkaline properties and apatite formation properties, which may provide a better environment for mineral uptake and reconstruction.3 Calcium silicate cement can provide the real and sustainable remineralization. As a result, the material could provide a remedy in cariology.

Conclusion: The calcium silicate cement was more effective than conventional glass ionomer cement and resin modified glass ionomer cement in remineralizing artificial caries affected dentin.

Keywords: Calcium silicate cement, Caries affected dentine, Glass ionomer cement, Remineralization, Resin modified glass ionomer cement

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postgraduate OC-00003

Effects of compressive stress combined with mechanical vibration on osteoclastogenesis in RAW 264.7 cells

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EXTENDED ABSTRACT

Background and purpose: There are many methods to accelerate tooth movement and there are numerous reports depict methods to speed the rate of orthodontic tooth movement by mechanical vibration. However, there are some limitations and controversial. This study is focused on mechanical vibration combined with compressive force applied in osteoclast precursor cells that mimic the environment in orthodontic treatment to investigate the osteoclastogenesis. gingiva.

Materials and Methods: RAW 264.7 cells were subjected to compressive force, mechanical vibration, or compressive force combined with vibration. The viability of cells was evaluated by Prestoblue assay. The osteoclast differentiation was investigated by tartrate-resistant acid phosphatase (TRAP) staining. *NFATc1*, *DCSTAMP*, and *CTSK* gene expressions were analyzed using real-time quantitative reverse transcription polymerase chain reaction.

Results and Discussion: Cell viability assays demonstrated mechanical stimuli in this study did not affect the viability. Matsuike et al., observed TRAP-positive multinucleated cells and an increase in the level of DC-STAMP mRNA expression of RAW 264.7 cells treated with 50 ng/mL of RANKL under 0.6 loading for 4 days1. In this study, combined compressive force and mechanical vibration significantly increased the numbers of TRAP-positive multinucleated cells (figure 1). Although vibration slightly increased NFATc1 expression, it did not significantly alter DC-STAMP or CTSK mRNA expression. Kulkarni et al., reported vibrations downregulated DC-STAMP gene and protein expression in osteoclast precursor cells2. Wu et al., also showed low magnitude, high-frequency vibration attenuated RANKLinduced upregulation of c-Fos in RAW 264.7 cells3. The c-Fos pathway plays an important role in the regulation of DC-STAMP expression, which may explain the decrease in DCSTAMP mRNA expression observed in the vibration group (figure 2).

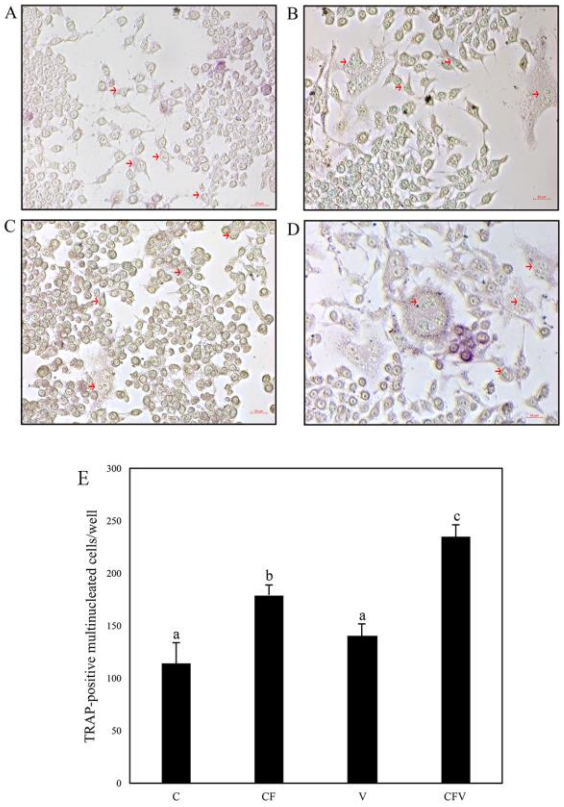

Figure 1: Combined compressive and vibratory force induces osteoclast differentiation in RAW 264.7 cells. The numbers of TRAP-positive MNCs (≥ 3 nuclei) with purplish-red color staining were counted (magnification = 40X, bar = 20 um). The red arrows indicate TRAPpositive MNCs. A: control group; B: compressed group; C: vibrated group; D: combined compression and vibration group; E: A greater number of TRAP-positive MNCs (≥ 3 nuclei) were observed in the combined group. Data are representative of three independent experiments. All values are shown as mean ± standard deviation. Significant differences between groups are indicated by different letters (a, b and c; $P < 0.05$, n = 3).

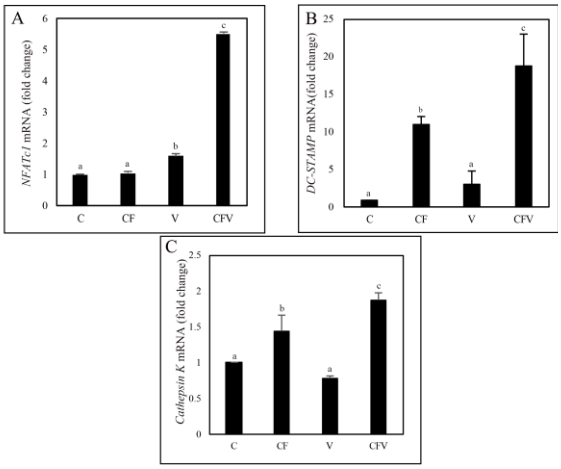

Figure 2: Effect of mechanical vibration combined with compressive force on mRNA expression in RANKL-treated RAW 264.7 cells. Real-time PCR analysis of A: NFATc1; B: DC-STAMP; C: CTSK. All data are mean ± standard deviation of triplicate experiments. Significant differences between groups are indicated by different letters (a, b and c; $P < 0.05$, n=3).

Conclusion: Compressive force combined with mechanical vibration had no effect on the viability of RAW 264.7 cells and increased *NFATc1*, *DCSTAMP*, and *CTSK* gene expression. Also, compressive force combination with mechanical vibration had additive effects on TRAP-positive MNCs (≥ three nuclei). These results provide more insight into the mechanisms by which vibratory force accelerates orthodontic tooth movement.

Competition

References:

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postgraduate OC-00004

Effects of Calcium Carbonate Microcapsules on Property of the Thermosensitive Hydrogel

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EXTENDED ABSTRACT

Introduction: The thermosensitive hydrogel system provides several advantages because it is easy to use and able to fill in the defects with irregular shapes and limited access while causing minimal injury to the patients.(1) Bone resorption associating with tooth loss increases a need for alveolar bone augmentation and bioactive bone substitutes to restore size and dimension of the alveolar bone to support forms and functions of the dental prostheses and dental implant placement.(2) Based on previous reports on osteoconductive and control release properties of the thermosensitive chitosan/collagen hydrogels(3) and the advantages of incorporating betatricalcium phosphate(4) and nano hydroxyapatite particles (nHA) in the thermosensitive hydrogels,(5) and calcium carbonate microcapsules ($CaCO_3$) exhibit an excellent biocompatibility and could be applied for different drug deliveries,(6) the current study incorporated the 2% nHA to improve osteoconductive property and mechanical strength of the chitosan/collagen hydrogel and added $CaCO_3$ to improve control release property of the nHAhydrogel. Therefore, the current study aimed to fabricate the thermosensitive $CaCO_3$-nHAchitosan/collagen hydrogel for promoting alveolar bone regeneration and investigate effects of the $CaCO_3$ on characteristics, cytotoxicity, and control release properties of the hydrogels.

Materials and Methods: The $CaCO_3$ were prepared by precipitating sodium carbonate that with calcium chloride in the carboxymethyl cellulose solution(7) and a natural flavonoid, quercetin (QT) was incorporated in the microcapsules during the precipitation process, then morphology of the microcapsules and released profiles of quercetin were examined using scanning electron microscope (SEM) and a total flavonoid content assay (TFC), respectively.(7) After that the thermosensitive 0%, 1% and 2% (w/v) $CaCO_3$-2% (w/v) nHA-chitosan/collagen hydrogels were prepared and the 10% (w/v) beta-glycerophosphate was used as an accelerator for the sol-gel transition under the temperature change at 37°C.(1, 3, 4) After that, the effects of the 0% - 2% $CaCO_3$ on the microstructures, mechanical and physical strengths, rheology and control release properties and cell cytotoxicity of the hydrogels were investigated. Cell viability assay was performed on the human osteoblast cell line (hFOB1.19, ATTC, USA) that were cultured with the culture mediums pre-incubated with the hydrogels.(3,4) The significant differences were set at $p<0.05$ (n=3-5, Mean±SD).immunofluorescence.

Competition

Results and Discussion: The $CaCO_3$-QT-microcapsules were round-shaped particles with rough surface size 2 – 5 µm with a rough surface and the microcapsules exhibited burst release of the QT in the culture medium. The thermosensitive $CaCO_3$-nHA-chitosan/collagen hydrogels changed from sol to gel at 37°C and the hydrogels had porous microstructures with an average porosity at 83.67±6.48%. The 2% $CaCO_3$-2% nHA-hydrogel exhibited a welldefined interconnected porous structure with pore size 128.07±34.81 µm. The incorporation of the 1% and 2% $CaCO_3$ into the 2% nHA-hydrogel significantly increased mechanical and physical strengths and viscoelasticity of the hydrogels, and improved control release profiles of the QT from the hydrogels. The findings demonstrated that the 1% and 2% $CaCO_3$ did not interfere with the gelation process of the thermosensitive-nHA-chitosan/collagen hydrogels and the 2% $CaCO_3$ enhanced the well-defined porous structure of hydrogel. It could be hypothesized that the distribution of the microcapsules into the gaps between the nHA particles in the matrix of the hydrogel, as demonstrated by the distribution of the small and large particles of the nHA and microcapsules on the pore walls, contributed to the increase of the mechanical strength, physical stability and viscoelasticity of the $CaCO_3$-nHA-hydrogels[4, 5] and the amount of the $CaCO_3$ at the 1% and 2% (w/v) did not overload the capacity of the nHAchitosan/collagen matrix to support the microcapsules in the hydrogels. The well-defined porous structure of the 2% $CaCO_3$-2% nHA-chitosan/collagen hydrogel and pore size larger than 100 µm would further provide a supportive microenvironment promoting matrix deposition and bone regeneration in the inner structure of the hydrogels.[1, 5, 8] Additionally, capacity of the $CaCO_3$-nHA-hydrogels to support cell growth and function as delivery vehicles for bioactive molecules was clearly demonstrated by the high degree of swelling (214.08±20.97%) and low degradation rate (15.24±5.83%), control released profiles of QT and non-cytotoxicity of the hydrogels.[5, 9] However, even though the $CaCO_3$ increased mechanical strength of the $CaCO_3$-nHA hydrogels, the compressive strength of the hydrogel at 4,974.83 ±354.34 Pa was still much lower than that of the cancellous bone at 0.1-16 MPa.[10] Therefore, the hydrogel is applicable for the well-defined 2-3 wall defects with the supporting tissue engineering membrane.

Conclusion: The 1% and 2% $CaCO_3$ microcapsules improved porous structure and increased mechanical strength, physical stability, and viscoelasticity of the thermosensitive 2% nHA-chitosan/collagen hydrogel. Based on the micro porous architecture, pore size, viscoelastic property, and cell cytotoxicity, the 2% $CaCO_3$-2% nHA-chitosan/collagen hydrogels exhibited the superior and optimal characteristics of a bioactive hydrogel for promoting bone regeneration in the non-load bearing area. Further studies would be performed to examine biocompatibility and alveolar bone regeneration capacity of the hydrogel in an animal model.

Keywords: Calcium carbonate microcapsules, Control release, Hydroxyapatite nanoparticles, Thermosensitive chitosan/collagen hydrogel, Bone regeneration

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postgraduate OC-00005

Identification of Contact Between Third Molar and Mandibular Canal by Deep Learning

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EXTENDED ABSTRACT

Introduction: The removal of the mandibular third molar (M3) may potentially impair the inferior alveolar nerve, which is situated in the mandibular canal (MC). This can be avoided by spotting risk signs that suggest contact between the M3 and MC on routine panoramic radiographs. However, noticing these signs can be challenging for general dental practitioners. Cone beam computed tomography (CBCT) is a better alternative modality since it reveals the structures clearly in three dimensions. Nevertheless, the machine is expensive and not widely accessible. Recently, deep learning (DL) has gained popularity in studying radiographic images. It has been reported in earlier research that DL was able to identify contact between the M3 and MC; however, CBCT images were not employed to verify the contact and augmented images were used.[1] While image augmentation is generally utilized to build a DL model; it is considered a computationally expensive process.[2] It was our interest to overcome these limitations and investigate if any differences in the diagnostic performances were discovered when no augmentation was performed.

Our study intended to build DL models from pre-trained convolutional neural networks (CNNs) without using image augmentation to identify contact between the M3 and MC on panoramic radiographs in which the contact was verified by CBCT images. The comparisons of the performances were studied among these DL models built with and without utilizing image augmentation and among various pre-trained CNNs.

Materials and Methods: This study was carried out following the approval of the Institutional Review Board from the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University (CoA no. 2021/079.0109). We gathered the panoramic radiographs from January 2013 to July 2021 from the Oral and Maxillofacial Radiology Clinic. We then divided the contact between the M3 and MC on the panoramic radiograph into three groups: contact, no contact, and misleading contact. The contact group displayed risk signs and contact on panoramic radiographs and CBCT images, respectively. The no contact group apparently displayed spaces between the two structures on panoramic radiographs. The misleading contact, which was not included in this study, displayed risk signs on panoramic radiographs but no actual contact between the structures on CBCT. The panoramic radiographs showing pathology, anomaly, or artefact were also excluded. Consequently, our panoramic radiographs comprised 1,365 individuals (1,800 M3) representing 900 M3 each in contact and no contact groups.

The panoramic radiographs were hand-cropped into image patches, focusing at the M3's root apices and MC, to the size of 70 x 70 pixels. These images were subsequently split randomly into train, validation, and test sets, obtaining 1,440:180:180 images, respectively.[3] Our DL models were built through FastAi library on Google Colabolatory, with the use of three pre-trained CNNs: AlexNet, GoogLeNet, and VGG-16. Each pre-trained CNN with 1,440 original images in the train sets was built without image augmentation. Then we increased the number of the train images in the multiples of five, obtaining four additional train sets, which consisted of 7200, 14400, 21600, and 28800 train images, and further built DL models using image augmentation. Therefore, five DL models were built per one pre-trained CNNs, and a total of 15 DL models were obtained. We measured the diagnostic performances of these models with the area under the receiver operating characteristic curve (AUC) and observed pairwise comparisons based on DeLong's method.[4]

Results and Discussion: The AUCs of the 15 models are presented in Table 1. These values went from 0.951 to 0.996, representing great performance in identifying contact between the M3 and MC. VGG-16 displayed the greatest performance when compared among DL models built without using image augmentation and was significantly better than GoogLeNet, possibly caused by the difference in the network architecture design of the pre-trained CNNs. When considering DL models built using image augmentation, VGG-16 displayed the most outstanding performance as well. The comparability between models built with and without image augmentation can be seen only in VGG-16, while the others—AlexNet and GoogLeNet—displayed significantly superior performances using image augmentation. As a result, the increase in the number of train images by augmentation did not influence VGG-16.

To the best of our knowledge, this study was one of the earliest studies that did not utilize image augmentation to build DL models to assess the M3 and MC. Without image augmentation, an efficient model could be obtained when using VGG-16 with enough original images as in our study. We used 1,440 original images in the train set. Moreover, our labels of the contact group were verified with CBCT images. The considerable number of our original images and the preciseness of our labels led to more superior AUC values than the earlier research.[1]

Table 1 Area under the receiver operating characteristic curve of the 15 deep learning models

Number of train images	Pre-trained CNNs		
	AlexNet	GoogLeNet	VGG-16
1,440 (original images)	0.956	0.951	0.981
7,200	0.994	0.982	0.990
14,400	0.995	0.990	0.996
21,600	0.989	0.987	0.992
28,800	0.988	0.985	0.992

CNNs; Convolutional neural networks

Conclusion: The diagnostic performances of the 15 models were excellent in identifying contact between the M3 and MC on panoramic radiographs. VGG-16 showed the most superior performance among models built with the equivalent level of augmentation. From the number of our original images (1,440 images), it may not be required to utilize image augmentation in VGG-16 to obtain an efficient model.

Keywords: Deep learning, Mandibular canal, Mandibular third molar, Panoramic radiograph

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postgraduate OC-00006

Periostin-Integrin Interaction Regulates Force-induced TGF-β1 and α-SMA Expression by Periodontal Ligament Cells

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EXTENDED ABSTRACT

Background and Objectives: Mechanical forces play essential roles in developing, repairing, regenerating, and modulating tissue homeostasis in various tissues, including the periodontal ligament (PDL). Periostin (POSTN) is one of the major matricellular proteins in periodontal ligament and was shown to be upregulated by mechanical force in human PDL cells. Moreover, *POSTN* knockout reduced the response of PDL cells to mechanical forces. Yet, how POSTN influences the effect of force on PDL cells is still unclear. Since it is known that POSTN interacts with αV-integrins, it is possible that POSTN-integrin signaling is required for the response of PDL cells to mechanical stimulation. Therefore, we aimed to investigate the requirement of POSTNintegrin signaling in force-induced expression of transforming growth factor-beta 1 (TGF-β1) and alpha-smooth muscle actin (α-SMA, a myofibroblast marker) in human PDL cells, since both are involved in the regulation of fibroblast-myofibroblast differentiation of connective tissue.

Methods: Human PDL cells were stimulated with an intermittence compressive force (ICF) for 24 hours in present and/or absence of POSTN and aV-integrins using RNA silencing technique. The quantitative real-time PCR and western blot analysis were used to determine TGF-β1 and α- SMA expression, together with scrape “wound” assay and immunostaining.

Results: Stimulation with an intermittence compressive force for 24 hours increased the expression of POSTN, TGF-β1, and α-SMA, along with increased SMAD2/3 phosphorylation. Knockdown of *POSTN* decreased the protein levels of TGF-β1, pSMAD2/3, and α-SMA responding to force stimuli. *POSTN* knockdown of human PDL cells resulted in delayed cell migration as determined by a scrape wound assay. However, migration was improved after

seeding these knockdown cells on pre-POSTN-coated surfaces. Finally, knockdown of αVβ5, but not of αVβ3, attenuated the compressive force-induced TGF-b1 expression.

Conclusion: The importance of POSTN-αVβ5 interaction in mechanical force-induced TGF-β1 signaling and involvement in force-induced α-SMA. These results support the critical role played by POSTN in maintaining tissue integrity and homeostasis of the periodontal ligament.

Keywords: Mechanical force, POSTN, Integrin, Periodontal ligament cells, α-smooth muscle actin.

postgraduate OC-00009

Satisfaction Level of Healthy Subjects on Mucoadhesive Film containing *Acmella oleracea* Extract

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EXTENDED ABSTRACT

Introduction: Mucoadhesive films have been introduced as a drug delivery system for topical treatment on mucosa including in the oral cavity. Due to the anesthetic effect of *A. oleracea*,[1] we hypothesized that its extract can be used to develop a safe mucoadhesive film for intraoral application, to relieve the pain associated with recurrent aphthous ulcer. The present study aimed to evaluate the satisfaction level of healthy participant on the usability of film containing *A. oleracea* extract.

Materials and Methods: This clinical study was conducted at the Dental Hospital, Faculty of Dentistry, Khon Kaen University and was approved by Center for Ethics in Human Research, Khon Kaen University on October 26, 2021. Mucoadhesive films containing *A. oleracea* extract were prepared under standard procedure of the film preparation. All films used in this study were from the same lot of preparation and kept in a vacuum cabinet at room temperature. The hazardous heavy metal contamination test was performed and certified safety by Central Laboratory (Thailand) Co., Ltd. Khon Kaen Branch.

A total of 36 healthy participants were recruited in this clinical study. Personal information and baseline data were collected using a questionnaire. Satisfaction levels toward the product's usability including convenience, appropriate duration of adhesion and suitability of taste, were collected using numeric rating scale (NRS) in self-reported questionnaire (online google form) at 24 hours after application.

Results and Discussion: The mucoadhesive film containing *A. oleracea* extract as the topical pain reliever has been successfully developed and tested for drug delivery properties in vitro by our interdisciplinary team at the Khon Kaen University. The crucial in vitro findings on drug releasing rate and adhering duration to the mucosa, were promising and suggested high potential for clinical use in management of recurrent aphthous ulcer (RAU). In this initial clinical study, healthy participants were recruited and the films had been applied on normal oral mucosa, in order to focus on the outcomes of the satisfaction on applying the film.

The average level of participants' satisfaction was as high as 8.83 on "convenient using". This finding demonstrate that the tested film was easy to use for intraoral application, consistent with other previous studies.[2,3] The contact period with oral mucosa has been suggested as another crucial parameter of drug delivery system.[3] As the result of this study, the average score on satisfaction level toward the "appropriate duration of adhesion" was 7.16, demonstrating that the intraoral adhesion duration was appropriate. Whereas the average score on the "suitability of taste" was rather low at 4, because of the bitterness derived from the releasing of *A. oleracea* extract. Data from the questionnaire also showed that many participants explained the experience of brief sweetness of the xylitol coated, occurring immediately after film application. Although the overall satisfaction on taste remained poor, there were relatively few participants who highlighted the importance of the flavor of the product. Anyhow, the taste will be one of the main characteristics to be improved as it could affected the overall clinical performance of the film.

Conclusion: The satisfaction of healthy adults in using the film containing Acmell oleracea extract on the normal oral mucosa were, at least, at the acceptable level, on several aspects including measurement of convenience and appropriate duration of adhesion. While the acceptability of the taste may need to be improved in the future. We are currently conducting a randomized control trial to evaluate the effectiveness of local anesthetic effect and safety of this film in the large numbers of healthy participants (Clinicaltrial.gov with NCT05219747 identifier).

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Effect of Orthodontic Loading Magnitude on the Periodontal Ligament Thickness: A Preliminary Study

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EXTENDED ABSTRACT

Introduction: Tooth autotransplantation is an important alternative for replacing missing teeth. However, this method is often perceived as unpredictable because of the possibility of irreversible replacement resorption or ankylosis. The preapplication of orthodontic loading has been shown to ease the extraction of the donor teeth and enhance the proliferation of the periodontal ligament (PDL), thus, avoiding risks of root damage and improving successful tooth autotransplantation.[1,2] However, little is known regarding the effects of various orthodontic loading magnitudes on the thickness of the PDL. Therefore, the purpose of the present study was to determine the changes in remaining PDL thickness on the root surface of extracted premolars following the application of orthodontic loading of different magnitudes.

Materials and Methods: Twenty premolars were divided into control and preloaded (0.014", 0.016", and 0.016 x 0.022" improved superelastic NiTi alloy archwire - ISW) (Sentalloy®, Tomy International, Inc., Tokyo, Japan) groups. Premolars were preloaded for 8 weeks before being extracted. Extracted premolars were stained with toluidine blue for the assessment of the remaining PDL thickness on the root surface. The extracted teeth were washed gently in phosphate buffered saline and fixed with a 10% buffered formalin solution for 24 h. Teeth were stained with toluidine blue. After staining, the radicular portion was sectioned into the apical, middle, and cervical thirds using a diamond disk. Each sample was sectioned and then digitally photographed under a stereomicroscope. Images were analyzed using ImageJ software and the stained was measured. (Figure 1) Data was collected and analyzed statistically.

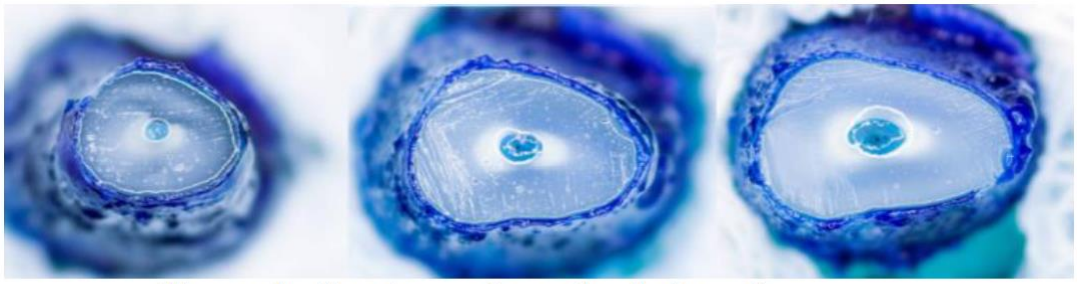

Figure 1. Sections of a preloaded tooth.

Results and Discussion: The preloading groups (0.014", 0.016", and 0.016 x 0.022" ISW) showed a significant increase in the overall PDL thickness compared to the control (0.09 ± 0.02 mm) ($p<0.01$). The 0.016 x 0.022" ISW group (0.224 ± 0.019 mm) provided the highest increase in overall PDL thickness compared to the control (2.5 times) ($p<0.01$). The 0.014" (0.128 ± 0.013 mm) and the 0.016" ISW groups (0.166 ± 0.005 mm) provided a significant increase in the PDL thickness compared o the

Competition

control of 1.4 and 1.8 times, respectively ($p<0.01$). Moreover, a strong positive correlation ($r = 0.951$) between the orthodontic loading magnitude and the increase in the PDL thickness was observed ($p<0.001$).

The preapplication of orthodontic loading results in increased proliferative and metabolic activities of the PDL cells.[1] Moreover, bone resorption through osteoclastic activity occurs creating irregular spaces in bone with the simultaneous increase in the PDL thickness. These cellular changes facilitate atraumatic extraction with reduced risks of PDL tissue damage.[1,2,3] Consequently, common complications such as root resorption and ankylosis following tooth autotransplantation can be eliminated.

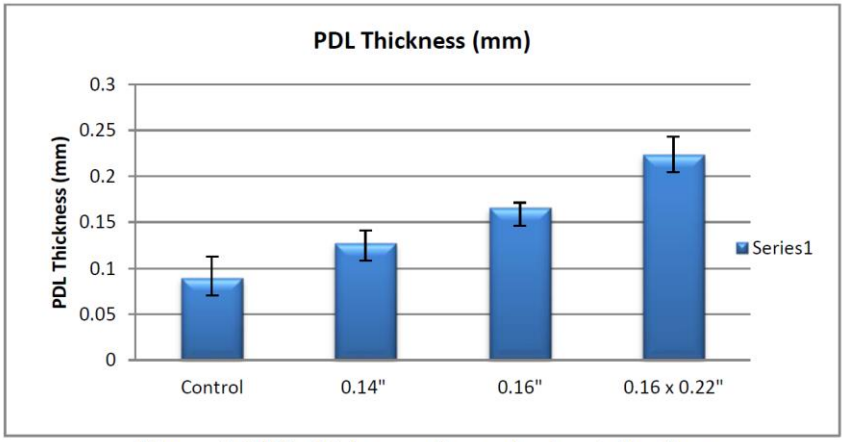

Figure 2. PDL thickness after orthodontic loading.

Previous studies investigated the duration of orthodontic loading on the enhancement of PDL.[2,3] A minimum period of 4 weeks of orthodontic loading duration for the enhancement of PDL and easy extraction.[2,3] However, the *in vivo* effect of different force magnitudes on PDL proliferation has not been investigated.

The present study demonstrated that the magnitude of orthodontic load played an important role in the increase in the PDL thickness. Moreover, a strong positive correlation between the orthodontic load and PDL thickness on the root surface of extracted premolars was observed. Consequently, predicting the required PDL thickness by controlling the orthodontic loading might become a useful approach for successful tooth autotransplantation.

To the author's knowledge, this study is the first comprehensive investigation of the relationship between orthodontic loading on the thickness of the PDL. Accordingly, we can consider that the results obtained in the present study are new finding and deserves further investigation.

A limitation of the present study was the reduced number of subjects and the possibility of intraindividual variation. Therefore, further studies with an increased number of samples must be performed.

Conclusion: The orthodontic loading magnitude is strongly correlated to the increase in the PDL thickness. The possibility of increasing the PDL thickness is advantageous for avoiding ankylosis and the increasing success of tooth autotransplantation.

Keywords: Orthodontic loading, PDL thickness, Tooth autotransplantation

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Proceedings

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Efficacy of Cleaning Solutions on Micro-Shear Bond Strength to Saliva Contaminated Zirconia

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ABSTRACT

Background and Objective: To observe the effect of different cleaning solutions on micro-shear bond strength (μSBS) between saliva contaminated zirconia and resin cement.

Methods: Forty-eight cylindrical discs of Cercon® ht zirconia, were embedded in metal molds and polished. After being contaminated with human saliva, all samples were separated into 8 groups at random using the following surface decontamination techniques: group 1 (NC): no contamination (control group), group 2 (WS) water-spray rinsing, group 3 (AA) sandblasted with aluminum oxide particle, group 4 (IC) applied with Ivoclean®, group 5 (SH) applied with sodium hydroxide, group 6 (SHC) applied with sodium hypochlorite, group 7 (EDT) applied with Ethylenediaminetetra acetic acid, group 8 (PA) applied with polyacrylic acid. Randomly, one sample from each group was chosen to have its surface morphology examined using surface elemental analysis with X-ray photoelectron spectroscopy (XPS). Multilink N resin cement was cemented on each specimen by injecting into polyethylene tubes (4 resin cement rods per sample). After being stored in distilled water at 37 ºC for 24 hours, specimens were subjected to a micro-shear bond strength test at a cross-head speed of 1 mm/min. Data was analyzed using one-way ANOVA, followed by the Tukey's test (p>0.05). Modes of failure were categorized by using the optical microscope.

Results: The highest significant μSBS was shown in SH group (29.25 ± 2.61 MPa) and the lowest significant μSBS was shown in WS group (19.31 ± 2.03 MPa). Failure mode analysis showed predominantly adhesive failure in WS and PA groups, while mixed and cohesive failure were found in the other groups. The elemental analysis showed the least detected carbon to zirconium ratio was in AA group.

Conclusion: There were statistically significant differences in μSBS between surface decontamination groups. Sodium hydroxide, Ivoclean®, and sodium hypochlorite were effective in restoring the μSBS between saliva contaminated zirconia and resin cement.

Keywords: Bond strength, Decontamination, Resin cement, Saliva, Zirconia

Proceeding

Introduction

All-ceramic materials with outstanding esthetic and mechanical qualities have been developed as a result of the growing need for esthetic restorations in dentistry.[1] Zirconia has numerous advantageous qualities, but in addition to these, it is challenging to connect to other structures due to its chemical inertness, high density, and lack of pores.[2] Studies have shown that sintered zirconia exhibited much higher adhesion loss compared to other all-ceramic materials.[3] The adhesion surfaces must also be kept clean before cementation in order to improve longevity of the restoration.[1,2,4]

Before being cemented, all prosthetic restorations must be tested in the oral environment. Saliva may have contaminated the restoration material, which frequently results in decreased resin cement bonding to zirconia.[5] As a result, after saliva contamination, the zirconia surface needs to be cleaned using mechanical or chemical means.[6] Numerous techniques, including silica coating, hydrofluoric acid, phosphoric acid, oil-free air streams, air-water spraying, ultrasonic cleaning, alcohol, and plasma treatment have all been tried and tested to clean the contaminated zirconia surface.[1,5] However, mechanical cleaning techniques like sandblasting can result in surface deformations, and chemical agents can alter the zirconia phase.[7] Nonabrasive universal cleaning agents have become more popular, but insufficient research has been done on their effects on zirconia's surface chemistry. Their benefits or drawbacks in comparison to mechanical or chemical cleaning methods, and how resin cement affect long-term bond strength.[1,8] Studies examining the bond strength between contaminated zirconia surfaces and resin cement are also scarce.[1] Therefore, the purpose of this in vitro study was to examine the effect of seven different surface cleaning methods to adhesive resin cement on the micro-shear bond strength (μSBS) of zirconia surfaces with combined contaminants. The null hypothesis stated that the various cleaning procedures and adhesive resin cements would have no impact on the contaminated zirconia μSBS.

Materials and Methods

The protocol of this study has been approved by the Faculty of Dentistry Human Experimentation Committee (No.24/2021). With the informed consent of the donor, saliva used was from a non-smoking male who had fasted for 1.5 hours before the saliva collection. All tests were carried out using fresh saliva. Ivoclean® (IC, Ivoclar Vivadent, Schaan, Liechtenstein, lot #: Z01GMJ), which contains zirconium oxide, water, polyethylene glycol, sodium hydroxide, pigments, and additives, was tested. Sodium hydroxide (SH, NaOH), sodium hypochlorite (SHC, NaOCl), EDTA (EDT, [CH2N(CH2CO2H)2]2), and polyacrylic acid (PA, CH2-CHCO2H) n, Mw 250,000 35%wt.) were diluted with distilled water into 1.0 %wt., 17.0 %wt., and 10 %wt. solutions respectively.

Using computer-aided design and production technology, a total of 48 cylindrical (10 mm diameter and 2-mm thickness) zirconium

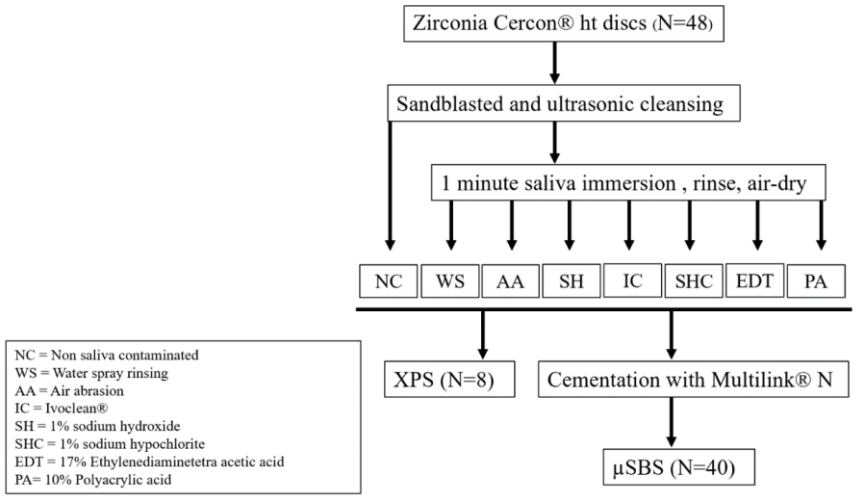

Figure 1. Design of this study.

oxide ceramic specimens (Cercon® ht A3, Dentsply Sirona, Bensheim, Germany lot #: I8042591) were created and thoroughly sintered in accordance with the manufacturer's specifications. All specimens were then embedded in acrylic resin, one surface of specimens was polished using silicon carbide (SiC) paper with decreasing abrasiveness to 1200 grit and sandblasted with 50-µm Al2O3 particles at 2.5-bar pressure for 10 s at a 10 mm distance. Subsequently, the specimens were ultrasonically cleaned in distilled water for 10 min. All samples were divided into eight research groups (Fig. 1). Except for the control group (group NC, no saliva contamination), all samples were submerged in saliva for one minute, rinsed with water spray for 15 seconds, and then dried by air for 15 seconds. In group WS, no further cleaning was performed. In group AA, specimens were air-abraded, ultrasonicated, rinsed, and air-dried as described above. In group IC, a micro-brush was used to apply IC, which was allowed to react for 20 seconds, followed by rinsing with water-spraying for 15 seconds and air-drying for 15 seconds, according to the manufacturer's recommendation. In groups SH, SHC, EDT and PA the corresponding solutions were applied with micro-brush and allowed to react for 20 seconds, respectively, rinsed, and air-dried in the same way as for group IC. The study design for surface analysis and micro-shear bond strength testing is illustrated in Fig. 1.

To determine the effectiveness of the cleaning methods, specimens of the eight test groups were examined with X-ray photoelectron spectroscopy (XPS). High resolution scans of carbon (C1s), oxygen (O1s), zirconium (Zr3d), nitrogen (N1s), aluminum (Al2p), and silica (Si2p) peaks were obtained. Ratios of C/O, C/Zr, O/Zr, N/Zr, Al/Zr, and Si/Zr were calculated.

For micro-shear bond strength testing, 40 zirconia disks in all were created and applied with 10-MDP Cesead® N Opaque Primer (Kuraray Noritake Dental Inc., Okayama, Japan; lot #: BF0030). Zirconia surfaces were treated according the study design (Fig. 1). Freshly-mixed Multilink® N (Ivoclar Vivadent, Schaan, Lichtenstein; lot #: Y26001) was applied to the surface by packing the material into cylindrical-shaped plastic matrices with an internal diameter of 0.8 mm height 0.5 mm and then irradiated for 20 seconds using a LED curing light (Bluephase® LED curing light, Ivoclar Vivadent, Schaan, Liechtenstein) with a soft start mode at 1,200 mW/cm2. In this manner, four bonded resin cylinders were made on one zirconia disk specimen and a total of 20 resin cylinders prepared for each group. All bonded specimens were kept in water at 37°C for 24 hours before being tested.

The specimens were perpendicularly engaged at their bonded resin cylinder bases with a round-notched custom loop wire in a universal testing machine (Instron 5566; Instron Ltd., Canton, USA) at a crosshead speed of 1.0 mm/minute until bonding failure occurred. Bond strengths (MPa) were calculated from the peak load of failure (N) divided by the bonded surface area. All shattered surfaces were examined under a 10x optical microscope after debonding to identify the failure mode: A, adhesive failure at the zirconia-resin interface; C, cohesive failure within resin; and M, a combination of these failure modes (mixed failure). In addition, an optical microscope was used to observe the debonded surfaces.

Data sets obtained for µSBS was first examined for their normality and analyzed with one-factor analysis of variance followed by post hoc Tukey's tests for pairwise comparisons. Statistical analyses were carried out using SPSS 25.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at $\alpha = 0.05$.

Results

Micro-shear bond strength and mode of failure are show on the Table 1 and Fig. 2 respectively. Group SH showed the highest shear bond strength value (29.25 ± 2.61MPa) but not significant different from groups IC and NC. Groups AA, IC, and SHC exhibited statistically similar bond strength values to that of group NC. In contrast, groups

Table 1. Mean micro-shear bond strength (MPa +- SD) of the test groups.

Group	Surface Decontamination	Micro-shear bond strength Mean ± SD
1	NC	27.36 ± 2.38^{CD}
2	WS	19.31 ± 2.03^{A}
3	AA	26.66 ± 2.62^{C}
4	IC	28.25 ± 2.31^{CD}
5	SH	29.25 ± 2.61^{D}
6	SHC	26.62 ± 2.04^{C}
7	EDT	22.18 ± 2.26^{B}
8	PA	22.35 ± 3.00^{B}

Different superscript letters indicate statistically differences. ($p<0.05$)

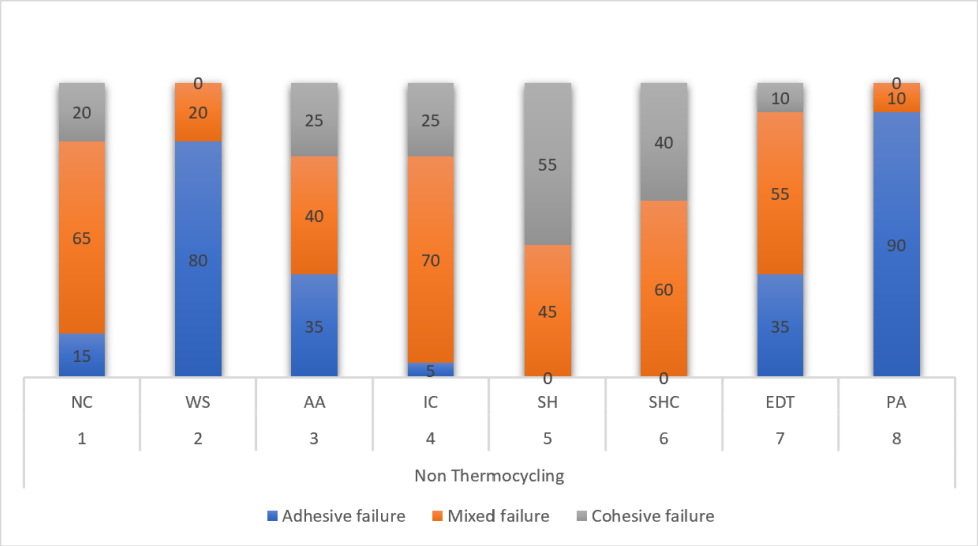

Figure 2. Percentage of failure mode.

EDT, PA, and WS showed significantly lower bond strengths than the aforementioned five groups (i.e., groups SH, NC, IC, AA, and SHC).

For groups NC, AA, IC, SH, SHC and EDT, mixed failures outnumbered adhesive failures. For groups PA and WS, the higher frequency of adhesive failures observed when compared to mixed failures.

The results of the XPS analysis are shown in Table 2, 3. The peak intensity ratios of C/O, C/Zr, and N/Zr were the highest in group WS (2.34, 2.86, and 0.31 respectively). After air-abrasion (group AA), the ratios were lowered, being comparable to those of group NC. The four cleaning solution groups showed C/O and C/Zr ratios that were similar to group AA—except for group PA, which exhibited notably higher ratios.

Discussion

The μSBS of contaminated zirconium was statistically different between various surface cleaning techniques. Therefore, this investigation rejected the null hypothesis.

As in similar studies[1], The findings of this study demonstrate the need for thorough cleaning of zirconia surfaces following contamination for long-lasting bond strength. Despite of the resin cement used, the μSBS was lower in group WS without a cleaning agent applied than in the other groups. This conclusion is also supported by the fact that the majority of the specimens in these groups had adhesive failure types. Contaminants occupying the oxide layer on the zirconia surface, which restricts the bonding connectivity, is the reason of the bond failure seen in the WS groups.[6,8]

The statistics confirm that the aluminum oxide sandblasting approach greatly promotes bond strength.[1,5,6] However, according to some research, this technique could result in cracks and deformations on the restored surface.[7] In the previous study's SEM imaging, it was discovered that the surface of the AA specimens had more surface deformation and microporosity than from other cleaning techniques. Based on this knowledge, it is clinically advised to employ the desired pressure setting and sand particle size within the acceptable ranges for zirconium material during sandblasting, particularly around the edges of zirconia restorations.[9]

The XPS results from the current investigation were consistent with those from related studies[10] and demonstrated that all surface cleaning techniques only partially cleaned the surface. In spite of the surface element ratios found after sandblasted show that this approach cleans more thoroughly, some pollutants were still present. One sign that surface cleaning cannot be performed is the presence of C element on the surface as a result of insufficient removal of potential saliva contamination. When the surface cleaning methods were compared, the highest C element ratios were observed in the WS, PA, then EDT groups. This matched the findings for binding strength for the cleaning techniques. The residual O element in the environment affects the bond strength and resin polymerization. Higher bond strength and more successful resin polymerization especially on surfaces with a low O/Zr ratio.[6] The group with the lower O/Zr ratio displayed the maximum bond strength, whereas the control group displayed the lowest strength. The PA method exhibited a higher O/Zr ratio and lower bond strength than did IC. As a result, the IC bond strength values were much higher than the PA values in terms of numerical value ($p < 0.05$).

According to the manufacturer, IC contains sodium hydroxide and is meant for extraoral use only. The four ingredients used to make the cleaning solutions have the potential to cause negative intraoral effects when they are present in high concentrations. 17% EDTA use as endodontic irrigant results in effective clinically result.

Table 2. Percentage value by weight of the elements detected on specimen surfaces after cleaning protocols.

Group	NC 1	WS 2	AA 3	IC 4	SH 5	SHC 6	EDT 7	PA 8
C	31.21	44.6	19.51	30.46	25.83	20.84	31.31	38.67
O	22.95	19.09	27.75	21.25	24.44	26.69	29.04	28.43
N	1.09	4.86	1.18	3.48	2.36	1.8	3.82	3.46
Al	17.37	13.31	21.41	15.01	8.54	10.5	10.81	8.78
Si	3.16	2.52	1.78	1.85	0	1.43	2.36	1.7
Zr	23.62	15.62	28.37	27.95	36.58	38.53	22.65	18.96

Table 3. Elemental ratios of carbon (C), oxygen (O), nitrogen (N), aluminum (Al), Silica (Si), and zirconia (Zr).

Group	NC 1	WS 2	AA 3	IC 4	SH 5	SHC 6	EDT 7	PA 8
C/O	1.36	2.34	0.70	1.43	1.06	0.78	1.08	1.36
C/Zr	1.32	2.86	0.69	1.09	0.71	0.54	1.38	2.04
O/Zr	0.97	1.22	0.98	0.76	0.67	0.69	1.28	1.50
N/Zr	0.05	0.31	0.04	0.12	0.06	0.05	0.17	0.18
Al/Zr	0.74	0.85	0.75	0.54	0.23	0.27	0.48	0.46
Si/Zr	0.13	0.16	0.06	0.07	0.00	0.04	0.10	0.09

Dentine conditioners often utilize 10% PA to improve the adhesion between dentine and glass ionomer cement. While 5.25% SHC is a frequently used tissue solvent, a solution of 1% SHC can effectively dissolve tissue. Experimental zirconia cleaning solutions with relatively low concentrations were made and tested with an aim toward intraorally use.

For group WS, the N element was detected on the zirconia surface. The N/Zr ratio was decreased after cleaning with the solutions, but N was still present on the zirconia surfaces. Only in groups NC and AA, where the surfaces were air-abraded, was nitrogen found. These results may suggest that air abrasion has a greater cleaning capacity for zirconia surfaces contaminated with saliva than either IC application or 1% SHC, even though there were no appreciable variations in shear bond strength between the three cleaning techniques. To determine whether cleaning solutions have a stronger long-term clinical bond than air abrasion, more investigation is still required.

In order to remove inorganic minerals from the smear layer and underlying dentin, endodontists frequently use the well-documented chelating agent EDT.[11,12] Group EDT, in contrast to groups NC and AA, displayed much lower bond strength. Similar to this, XPS analysis revealed that group EDT had a greater C/Zr ratio than group SHC (1.38 vs 0.54). These results suggest that 17% EDT solution removes carbon less completely than 1% SHC. Therefore, it appears that the treatment is useless in removing the saliva-damaged zirconia surface.

Indicated to remove unwanted residue, change wetting capacity, and improve glass ionomer cement material adaptation to dentin, PA solution is frequently used to remove smear layer and surface contaminants while also changing surface energy and exposingthe mineralized dental structure for acid and ionic diffusion.[13,14] Therefore, the chemical interaction between calcium ions in the tooth and carboxyl groups of polyacrylic acid is what causes the adhesion process. 10% PA cleaning efficacy was found by XPS analysis to be relatively ineffectual, and group PA had the lowest bond strength value among the test groups. It's possible that this is caused by chemical forms on the zirconia surface that contain carboxyl groups from polyacrylic acids and interfere with the chemical link between the oxide layer and 10-MDP.

The manufacturer claims that the alkaline suspension of zirconium oxide particles in IC eliminates salivary phosphate impurities by adsorption (Ivoclar Vivadent Scientific Documentation, 2011). P element from XPS was not detected in any experimental group, so this study could not confirm the mechanism that manufacturer claimed. However, it seems that the use of IC created a clean surface for enhanced resin bonding by successfully removing impurities from the surface, despite of salivary phosphate. Group SH which also contains of 1% sodium hydroxide same as the commercial product IC achieve the highest shear bond strength in this study which means that cleaning with isolate 1 % sodium hydroxide solution can restore shear bond strength to saliva contaminated zirconia. Sodium hydroxide which is strong alkaline solution can remove the organic substances on the saliva contaminated zirconia entirely. The XPS test also confirm with C/Zr and O/Zr ratio also tremendously reduce compared form the negative controlled group.

The results of our study that the group using SHC provide the bond strength similar to group using IC, AA and group without saliva contaminated (NC) are in agreement with previous study[6] According to the results of XPS analysis, zirconia surface cleaned with SHC had a lower O/Zr ratio similar to the group using SH which obtained the highest bond strength. The lower bond strength of group SHC than that of group SH in our study is surprising. This may be due to residual SHC may cause resin polymerization to be hindered by oxygen production.[15] The outcomes for group AA also proof that zirconia surface contaminated by saliva can be mechanically cleaned effectively using air abrasion.

When compare our investigation with a previous similar study[6], the results in gr NC, WS, AA, IC, and SHC of our study, the greater bond strength were found. Due to the loading technique, zirconia specimen, type of resin cement and specimen dimensions may be factors influencing the different shear bond strength between both studies. Micro-sized specimens was more recommended for shear testing because of higher bond strength and incidence of adhesive failures were observed with smaller bonded areas.[16]

Conclusion

The results of this investigation demonstrate that air-abrasion is an effective cleaning technique and that saliva contamination greatly affects the resin's ability to shear bond to zirconia. But a straightforward application of IC, 1% SH, and 1% SHC successfully eliminated the saliva impurities and created a clean surface. The chemical identification of the zirconia surface with XPS provided confirmation for the resin bond strength findings. To determine the effectiveness of cleaning solutions in enhancing the resin bonding of saliva-contaminated zirconia, however, long-term clinical investigations are still necessary.

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MRI Evaluation on Articular Disc Position after Mandibular Setback: A Pilot Study

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ABSTRACT

Background and Objective: Condylar displacement could be an unfavorable complication of sagittal split ramus osteotomies. Malposition of condyle leads to immediate relapse of occlusion and temporomandibular disorder after the surgery. Many studies showed changing of condylar position, condylar remodeling, and condylar resorption existed after orthognathic surgery correction for class III dentofacial deformities but its impact on articular disc position is still inconclusive. Therefore, we aimed to compare the preoperative articular disc position to the postoperative arrangement using magnetic resonance imaging (MRI).

Methods: We recruited 10 subjects with skeleton class III deformities who underwent orthognathic surgery at the Faculty of Dentistry, Chiang Mai University. Clinical examination and MRI scans were performed preoperatively, 3-month, and 6-month postoperatively. Articular disc positions depicted on the MR images were evaluated over the three phases.

Results: MR images analysis revealed different mean values of articular disc position angle among the three periods. At the 3-month phase after surgery, the articular discs located posteriorly to its preoperative positions. Then, they displaced anteriorly at 6 months after surgery. However, significant difference was not found (p=0.056).

Conclusion: The pilot study showed the articular disc positions displaced posteriorly to the preoperative locations and their relationships with condylar head tended to alter into less severity stage of internal derangement.

Keywords: Dentofacial deformities, Magnetic resonance imaging, Orthognathic surgery, Temporomandibular joint disc

Introduction

Bilateral sagittal split osteotomy (BSSO) has advantages in the large contact area of cancellous bone which promotes bone healing and the intraoral approach produces less scarring.[1] Condylar displacement could be an unfavorable complication of BSSO. Malposition of condyle leads to immediate relapse of occlusion and temporomandibular disorder (TMD) after the surgery.[2] Many studies had reported condylar changes after surgical correction of facial deformities. A systematic review showed that condylar remodeling and condylar resorption existed after orthognathic surgery for class III dentofacial deformities.[3] On the other hand, the articular disc position after surgical correction in class III dentofacial deformities is still controversial although disc-condyle relationship is an important parameter in the temporomandibular joint (TMJ) morphology and symptoms. Up to the present time, no systematic reviews have been concluded regarding this issue. Therefore, this pilot study is aimed to compare preoperative to postoperative articular disc position in skeleton class III patients who undergo the BSSO using magnetic resonance imaging (MRI) evaluation which currently is a gold standard imaging for TMJ soft tissue evaluation, especially the relationship between articular disc and condyle.[4-6]

Materials and Methods

The study was performed as a prospective observational study and the study design was approved by the Institutional Review Board, Faculty of Dentistry, Chiang Mai University (no. 11/2021) and has been registered in Thai Clinical Trials Registry (TCTR20220318002). We recruited patients with skeleton class III (the diagnosis was made by oral maxillofacial surgeons based on Steiner analysis.[7]), age range 18-35 years old, who received combined orthodontic and orthognathic treatment between August 2021 to June 2022 from the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand. The patients received BSSO for mandibular setback either in one or two jaw surgeries. The exclusion criteria were as follows; a, patients who received posterior bending surgical technique; b, patients with medical conditions that might affect bone healing process (i.e., diabetic mellitus, currently on bisphosphonate, steroid therapy); c, patients who are currently smoking; d, patients with history of condylar fracture and/or trauma; e, patients with a history of TMDs diagnosis or treatment prior to being included in the study; f, patients who present postoperative BSSO complications (i.e., condylar displacement, immediate postoperative occlusal instability, malunion or non-union bone segment); g, patients who met any contraindication for MRI examination. Lost follow-up and/or poor quality of MR images resulted in the discontinuation of these subjects.

MR scan and images analysis

MRI scans were performed by experienced radiological technologists using Philips Ingenia 1.5T MRI scanner (Philips Healthcare, Amsterdam, Netherlands). The images were conducted in three views as follows;

1. Axial view was conducted for locating condyles

2. Sagittal view was obtained when the patient is in painless maximum mouth opening. Bite block was placed in between the maxillary and mandibular incisors to comfort the patient and to help maintain the open-mouth distance during scanning. The image was used to observe disc-condyle relationship and reduction ability.

3. 3D image of TMJ was obtained when the patient is in a close mouth position. The image was used to observe disc-condyle relationship in the resting position.

The MRI parameters of each view were set as Table 1.

Table 1. MRI parameters.

Parameter	Axial View T2WI-TSE	Sagittal View PDWI with O-MAR	3D PDWI with O-MAR
Repetition time (TR)	5,290 ms	2000 ms	2000 ms
Echo time (TE)	120 ms	20 ms	28 ms
Field of view (FOV)	100 x 100 mm	100 x 100 mm	220 x 220 mm
Slice thickness (mm)	3	2	1
Matrix reconstruction	640 x 640	256 x 256	576 x 576
Flip angle (FA)	90°	90°	90°

3D, three-dimensional image; O-MAR, metal artifact reduction for orthopedic implants; PDWI, proton-density weighted imaging; T2WI-TSE, T2-weighted imaging turbo-spin echo.

The MR images were anonymized and divided into 3 groups according to the scan period. Each group had its order shuffled to minimize observer bias. An oral and maxillofacial radiologist with 5 years of experience who was blinded to the patient's history and clinical information was obligated to select and evaluate the most obvious articular disc-condyle relationship in MR closed-mouth sagittal view using RadiAnt Dicom viewer (Medixant, Promienista, Poznań, Poland). The variables were as follows:

1. Articular disc angle

We adapted the articular disc angle measurement from Kawakami et al.[8] (Fig. 1). The angle was measured between the deepest point of articular (glenoid) fossa and the distal part of articular disc. The positive (+) angle represents the posterior band locating anteriorly to the deepest point of the articular fossa while the negative (-) angle symbolizes the posterior displacement of the articular disc to the deepest point of the articular fossa.

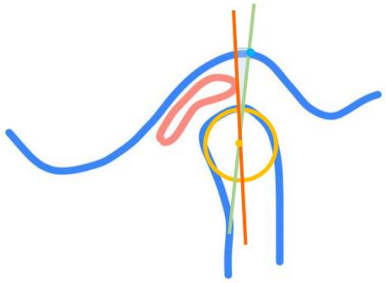

Figure 1. Articular disc position angle measurement modified from Kawakami et al.[8] The blue line shows the outline of TMJ structure including articular disc, condyle, articular fossa and articular eminence. The green line was drawn from the center of condylar head (yellow dot) to the deepest point of articular fossa (blue dot). The red line was drawn from the center of condylar head to the distal part of articular disc (pink outline). The blue area represents the articular disc angle.

2. Disc-condyle relationship

The observer evaluated disc-condyle relationship in MR sagittal view in both maximum open mouth and close mouth positions and classified the relationship into 5 categories[9] as follows; 1. normal disc position; 2. partial anterior disc displacement with reduction (PADwR); 3. partial anterior disc displacement without reduction (PADwoR); 4. complete anterior disc displacement with reduction (CADwR); and 5. complete anterior disc displacement without reduction (CADwoR) (Fig. 2).

Surgical procedure

All patients underwent the surgical mandibular setback using Modified Epker BSSO technique under general anesthesia by experienced oral and maxillofacial surgeons at the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chiang Mai University. The surgeons were acknowledged the surgical-related information to perform an appropriate treatment and adjust the confounding factors, but they were blinded from the patient's MRI results. According to surgical digital planning. The amount of bilateral mandibular setback was ranged from 3 - 16 mm (mean 8.30 ± 3.72 mm). In the operation, condylar head was manually positioning near the centric relation in the articular fossa. Semi-rigid internal fixation with titanium miniplates and miniscrews were attached between two segments of mandible. Postoperative intermaxillary fixation was remained for 2 to 4 weeks. Wafer splint was removed at one month after surgery and postoperative orthodontic treatment subsequently began. Patients were appointed as periodic follow-up for clinical evaluation and MRI examination of TMJ at three and six months after surgery. The radiologist was responsible for MR evaluation of articular disc angle and disc-condyle relationship as previously described.

Statistical Analysis

Characteristic data of subjects, timing of MR scans was described by mean ± standard deviation (SD). Since there was a skewed distribution, median value was used to represent articular disc position angle of each group. The different of the articular disc position angle between three periods was analyzed using Friedman two-way ANOVA. Statistical significance was considered if *p*-value was less than 0.05 at 80% power of test. The changes in categories of disc-condyle relationship were described and presented as bar graph.

Results

Ten patients (6 males and 4 females, average age 24 ± 4 years old) were included in this study and underwent an MRI scan at the time before surgery (mean 11 ± 9 days), 3-month after surgery (mean 97 ± 13 days), and 6-month after surgery (mean 190 ± 13 days). Three from twenty TMJs were excluded because of excessive metal-related artifacts in their MR images. Therefore, seventeen TMJs remained for further analysis.

Articular disc position angle

Median of articular disc position angle in preoperative group, 3-month postoperative group and 6-month postoperative group were 49.077, 36.398, and 41.931 respectively. There was no statistically significant difference (p=0.056) between three groups. Median of articular disc position angle was reduced at 3-months post-surgery, then increased at 6-months post-surgery (Fig. 3).

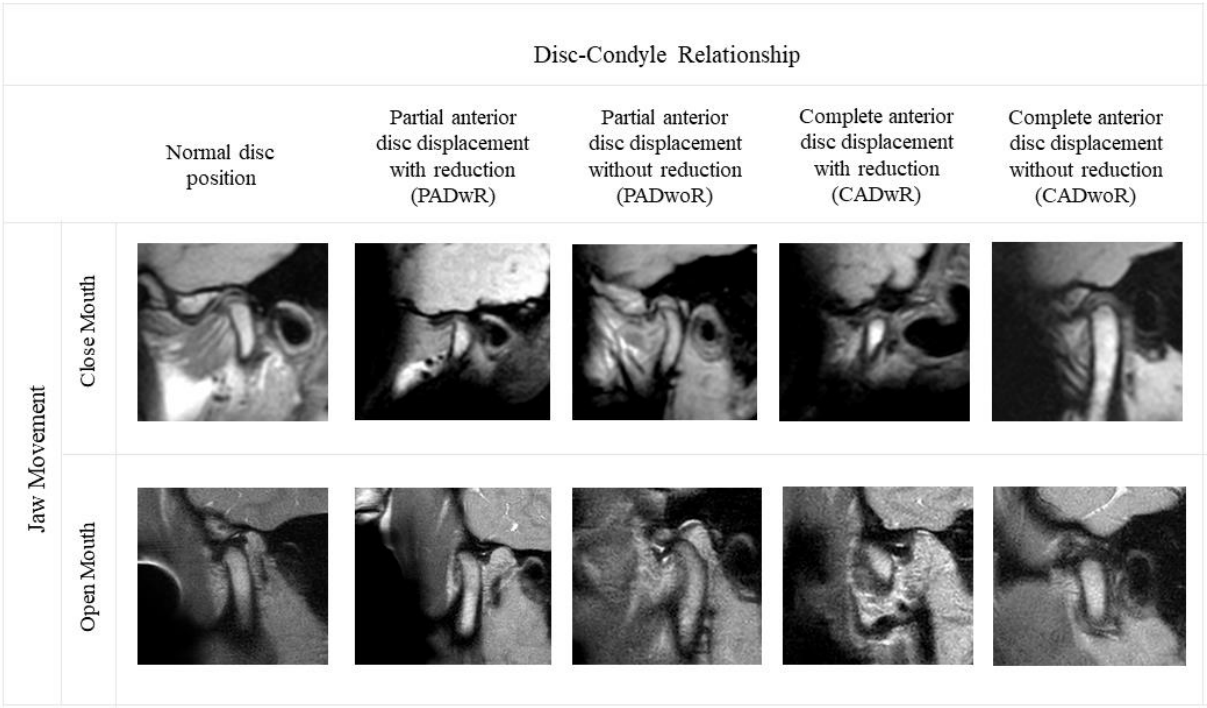

Figure 2. Showed the pictures of the sagittal disc-condyle relationship in open mouth view and close mouth view.

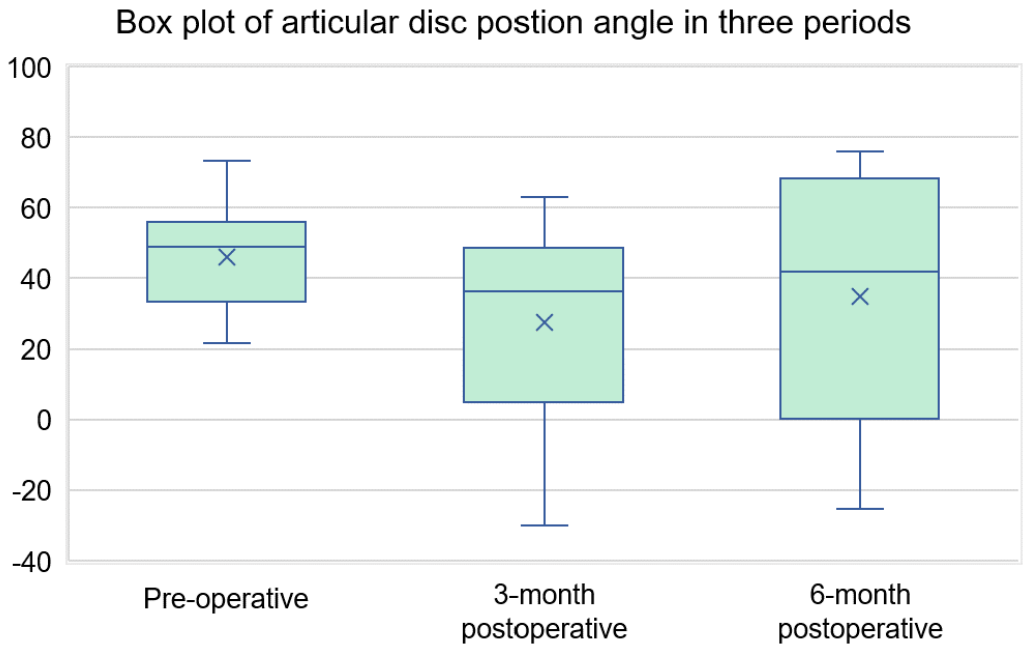

Figure 3. The box plot showed data distribution of articular disc position angle in the preoperative group, the 3-month and the 6-month postoperative groups.

Disc-condyle relationship

Apart from the reduced angle of the articular disc, disc-condyle relationship was also changed (Fig. 4). Before surgery, normal disc-condyle relationship was found in only one case, which then increased to six cases and five cases at 3 months and 6 months post-surgery, respectively. PADwR reduced from nine to three cases at 3 months post-surgery, then increased to six cases at 6 months post-surgery. Four from six cases of CADwR shifted to normal disc-condyle relationship and PADwR. At the times after surgery, no CADwoR was found.

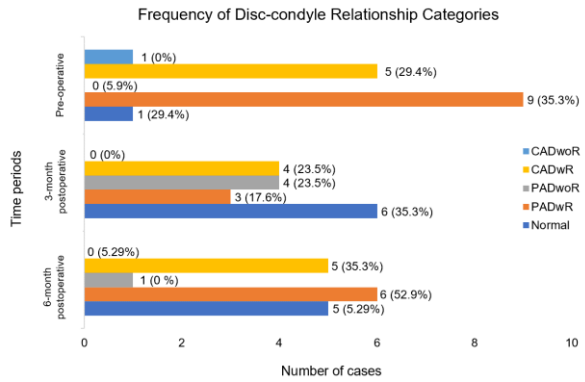

Figure 4. Bar chart presents the frequency of disc-condyle relationship categories of the preoperative group, the 3-months and the 6-months postoperative groups. CADwoR, complete anterior disc displacement without reduction; CADwR, complete anterior disc displacement with reduction; Normal, normal disc position; PADwoR, partial anterior disc displacement without reduction; and PADwR, partial anterior disc displacement with reduction.

Discussion

Skeleton class III relationship was majority cases of orthognathic surgery done in Asian population.[10-12] This study aims to find a difference between preoperative and postoperative articular disc position in skeleton class III patients who underwent surgical setback BSSO. The results revealed difference in mean values of the articular position angle between groups. However, statistical analysis showed no significant differences.

Drace and Enzmann[13] defined normal position of articular disc position 12 o'clock position relative to the condyle and considered 0°–10° from this position as normal disc-condyle relationship. Ögütcen-Toller et al.[14] thoroughly classified the disc displacement degrees of 11°-30° as slight anterior displacement and those of 31°-50° as moderate anterior disc displacement. In this present study, all postoperative periods showed mean value of articular disc position angle were higher than 10°. Moderate anterior disc displacement was shown preoperatively and in 6-months postoperatively. Meanwhile, in the 3-month postoperative period, the mean value of the articular disc position angle was considered as slight anterior displacement. The position of articular disc related to condylar head was displaced posteriorly compared to its preoperative position and had a tendency to regain its original position after a six-month period.

This finding related to disc-condyle relationship categories as the normal relationship was the most prevalent category at 3 months post-surgery, but for 6 months post-surgery the most frequent relationship was anterior disc displacement with reduction. The result is discussed in two issues as follows:

1. Osseous changes of temporomandibular structure after orthognathic surgery

Like the other joints in body, Biomechanics of TMJs depend on co-ordination between the osseous and soft tissue structures. Change in one structure will alter others. In the previous studies[15-21], many techniques of imaging revealed changes in osseous structure of TMJs after BSSO, including posteriorly position in glenoid fossa, inward rotation of condylar head, and condylar head remodeling.

Articular disc is attached to the condyle with discal ligament so when the condyle has moved posteriorly, articular disc will consequently move. Our finding is similar to Lee et al.[22] They divided the subjects into 3 categories by disc-condyle relationship. Their results showed that every group of subjects had posterior position of articular disc in relative to glenoid fossa at 3 months after surgery. Meanwhile, Firoozei et al.[23] studied the effects of BSSO in skeleton class II patients with a similar MRI analysis method performed by Lee et al. The results presented conversely. They also concluded that the articular discs tended to move anteriorly at 3-month after surgery. In long term follow-up, Kawakami et al.[8] found anterior displacement of articular disc at 1 year after setback BSSO. Similar to our finding, disc position was prone to displace anteriorly as time went by. However, studies about condylar positional changing still reported posterior position compared to preoperative position at 6 months after BSSO. It is suggested that the articular disc position would not depend on the osseous structure only.

Lateral pterygoid muscle (LPM) possibly involves owing to its insertion on the medio-anterior part of the articular disc and constantly pulls the articular disc anteromedially by its tonicity. Normal morphology of articular disc and healthy retrodiscal tissue provide a proper position of the articular disc in disc-condyle complex. If the condition is deviant, disc displacement could occur.[24] Hasan et al.[9] observed thickening of LPM and elongation of retrodiscal tissue. They found that these factors are significantly associated with the degree of TMJ internal derangement. This study also reported that the higher degree of anterior disc displacement increased the odd ratio in relation to the posterior position of condyle in glenoid fossa.

2. Normal skeleton relationship and Occlusal stability

Occlusal instability and skeleton deformities can play a role as factors in TMDs[4] therefore, skeleton relationship correction treatment as orthognathic surgery should improve TMD status. Many studies revealed an improvement in TMD symptoms after orthognathic surgery. Kersten et al.[25] found 16.2% of 480 patients with different dentofacial deformities had pre-existing TMD symptoms, and that 66% of the patients tend to improve after surgery. The meta-analysis study assembled with 29 studies also found that TMD symptoms of skeleton class III patients were significantly reduced after orthognathic surgery.[26] The previous findings match with the result of this study, that shows 8 of 17 TMJs shifting into a less severe stage of internal derangement and only 3 TMJs had more severed. These can be a reason why TMD symptoms improved in previous studies, as disc-condyle relationship is an important parameter in TMJ morphology and symptoms. However, this study did not study correlations between the change in disc-condyle relationship and improvement of TMD symptoms, which should be the subject of future research.

Regarding the artifact, Cassetta et al.[27] studied the effects of metal orthodontic appliances in 3-Tesla MR images and they found image distortion in most areas of cranial and cervical regions but not in TMJ region. They suggested that debonding of stainless-steel brackets is not necessary when MRI was performed for purpose of TMJ evaluation. In this present study, Orthopedic Metal Artifact Reduction (O-MAR) technique which is widely used in case of an orthopedic implant or joint reconstruction cases was applied to help reduce the artifacts from fixed orthodontic appliances. There are uncontrolled limitations related to individual factors such as anatomical structures and appliances of orthodontic miniscrew implants. These factors resulted in artifacts' involvement with the TMJ resulting in the subject exclusion. Rejection to research participation after surgery was also incontinent, possibly due to either the COVID-19 pandemic or personal reasons.

Conclusion

In this present study, the articular disc was in a posterior position at 3 months after surgery and the disc tended to displace anteriorly at 6 months after surgery. The result of angle measurement was in accordance with the sagittal disc-condyle relationship. The disc also moved to a less severe stage of internal derangement after a short period of time. This finding can be implied that BSSO combined with

orthodontic treatment has no worse effect on TMJ biomechanism in skeleton class III patients.

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OM-00003

Salivary Glucose in Patients with Type 2 Diabetes Mellitus: A Preliminary Study

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ABSTRACT

Background and Objective: Saliva has been recognized as a valuable source of biomarkers that could reflect both oral and systemic conditions. This pilot study was aimed to investigate the potential of salivary glucose as a biomarker of glycemic status in Thai patients with type 2 diabetes mellitus (DM).

Methods: A total of 30 Thai subjects consisted of 15 healthy individuals (control) and 15 patients with type 2 DM were recruited. Serum and unstimulated whole saliva of each subject were collected in the same day after 8-hour fasting period for the evaluation of fasting plasma glucose (FPG), glycated hemoglobin (HbA_{1C}), and salivary glucose levels. Glucose assay based on glucose oxidase-peroxidase reaction was used to measure salivary glucose levels. Mann-Whitney U test was applied to compare blood glucose and salivary glucose levels between the control and the DM groups. Pearson correlation was used to analyze the correlation between salivary glucose and blood glucose levels.

Results: Salivary glucose level was significantly increased in the DM group compared to the control group. The level was correlated with HbA_{1C} ($r=0.405$, $p=0.026$) whereas a non-significant positive correlation between salivary glucose and FPG was revealed ($r=0.079$, $p=0.679$).

Conclusion: Salivary glucose level correlated with HbA_{1C} and demonstrated a potential to be a biomarker of glycemic status in patients with type 2 DM.

Keywords: Biomarker, Salivary glucose, Type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by persistent hyperglycemia. If persistent hyperglycemia lasts for a long time, other organs could be damaged and may develop various oral health problems including periodontal disease, dental caries, xerostomia, candida infection, and dysgeusia.[1,2] In 2035, it is expected that the percentage of Thai people diagnosed with DM would increase by 1.1 million.[3] Thus, DM screening is vital because it could prevent the development of the disease and help people to be aware of self-glucose-level. Until now, DM screening and monitoring has been relied on blood sampling which is painful. If there are more optional methods to measure glycemic status in a non-invasive way, it will be beneficial for the medical field.

Saliva has been recognized as a mirror of the body health.[4] Recently, various studies found that the biochemical composition in saliva could be used as biomarkers of some systemic and oral diseases including DM and periodontitis.[5] Some proteins and cytokines could be detected in both blood and saliva.[6,7] Salivary glucose has been proposed to be a biomarker of glycemic status since many studies reported significant correlation between blood glucose and salivary glucose levels in patients with DM.[8-12] The possible mechanism of this phenomenon may be due to the increased permeability of blood vessels and basement membrane of salivary gland in diabetic patients. Glucose which is a small molecule then can be diffused through the membrane and presented in saliva and gingival crevicular fluid.[13,14]

Although many studies reported the potential of salivary glucose as a glycemic biomarker, some studies showed contradicted results which may be due to the difference in study population and salivary glucose measurement methods.[13,15] In addition, there is no report with respect to salivary glucose measurement in Thai population. Therefore, this cross-sectional case control study aimed to investigate the relationship between salivary glucose and blood glucose levels in Thai population.

Materials and Methods

Study Subjects

The total of 30 subjects who attended the outpatient clinic at the King Chulalongkorn Memorial Hospital were recruited in this study. They were divided into 2 groups, the control group consisted of 15 systemically healthy subjects and the DM group consisted of 15 patients who were previously diagnosed with type 2 DM by physicians according to the classification and diagnosis of diabetes guideline, American Diabetes Association (ADA) 2022.[16] All subjects with type 2 DM were treated either by hypoglycemic medications or insulin injection. Prior to research commencement and subject enrollment, the study protocol was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University (IRB No.100/57) and the Ethic Committee of Srinakharinwirot University (SWUEC-005/2565F).

The inclusion criteria for healthy subjects are not having any medical conditions or taking any medicine due to illness, not having antibiotics or steroids during 3 months before enrolling this study. The DM group comprised of patients who were diagnosed with type 2 DM with no history of having antibiotics or steroid during 3 months before enrolling this study. Exclusion criteria for both groups were those who had salivary gland pathology or history of receiving radiation therapy in head and neck area, smoker, pregnant, lactating mothers, and alcoholism.

Clinical and Laboratory Experiments

To collect the data for clinical experiment, serum and unstimulated whole saliva were collected in the same day after 8 hour-fasting condition. Six milliliters of venous blood samples were collected from each subject by venipuncture at antecubital fossa for the measurements of fasting plasma glucose (FPG) and HbA_{1C}. FPG levels were evaluated using Alinity c glucose reagent kit (Abbott GmbH & Co., KG, Germany) that based on hexokinase/glucose-6-phosphate dehydrogenase (G-6-PDH) method. Percentage of HbA_{1C} levels were determined based on enzymatic method using Alinity c hemoglobin A1C reagent kit (Abbott GmbH & Co., KG, Germany). Both FPG and HbA_{1C} analyses were performed by the laboratory unit of the King Chulalongkorn Memorial Hospital.

The unstimulated whole saliva was collected using the spitting method. Briefly, each subject was asked to spit the saliva approximately 5 mL into a sterile tube which was placing on ice. Protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany) was then added into the tube immediately after saliva collection was completed. Saliva samples were centrifuged at 10,000g, 4°C for 10 min. to collect only the supernatant which was further aliquoted and stored at -80°C.

Salivary glucose concentration was analyzed using glucose assay kit based on oxidase- peroxidase reaction (EnzyChrom™, EBGL-100, BioAssay Systems, CA, USA). The assay was performed according to the manufacturer's recommendation. Briefly, glucose standards were prepared at the concentration of 0-30 µM to generate standard curve. Twenty microliters of standards and undiluted saliva samples were added into a black 96-well plate (Merck KGaA, Darmstadt, Germany). The working reagent which comprised of assay buffer, enzyme mix, and dye were further added into each well with the volume of 80 µL and the plate was incubated in the dark at room temperature for 30 minutes. The absorbances were measured at $\lambda_{ex/em}$ = 530/585nm using fluorescence microplate reader (Synergy H1 Hybrid Reader, Agilent, Santa Clara, CA, USA). Glucose concentrations (µM) of samples were analyzed using standard curve organized by Gen5TM data analysis software.

Statistical Analyses

Statistical analyses were performed using SPSS 25.0 software (SPSS Inc., Illinois, USA). The normality of all continuous data was analyzed using the Shapiro-Wilk. The chi-squared test was applied to analyze the categorical variable. Comparisons of characteristics between the control and the DM groups were performed using independent T-test or Mann-Whitney U test where appropriate. Pearson correlation was used to test the correlation between salivary glucose, HbA_{1C}, and FPG levels. The statistically significant difference was determined at $p<0.05$.

Results

Characteristics of Study Subjects

The characteristics of the study subjects were depicted in Table 1. The subjects in the DM group were older, had higher body mass index (BMI), higher fasting plasma glucose (FPG), and higher HbA_{1C} compared to the healthy control group. No significant difference in gender between the two groups was found.

Salivary glucose Measurement

Significantly higher salivary glucose level was revealed in the DM group compared to those of the control group ($p<0.0001$) (Table 1). The salivary glucose level could not be detected in 4 out of 15 healthy subjects (26.7%).

Correlation between salivary glucose, HbA_{1C}, and fasting plasma glucose levels

Salivary glucose level was significantly correlated with HbA_{1C} ($r=0.405$, $p=0.026$) (Fig.1a). Non-significant positive correlation between salivary glucose and fasting plasma glucose was found ($r=0.079$, $p=0.679$) (Fig. 1b).

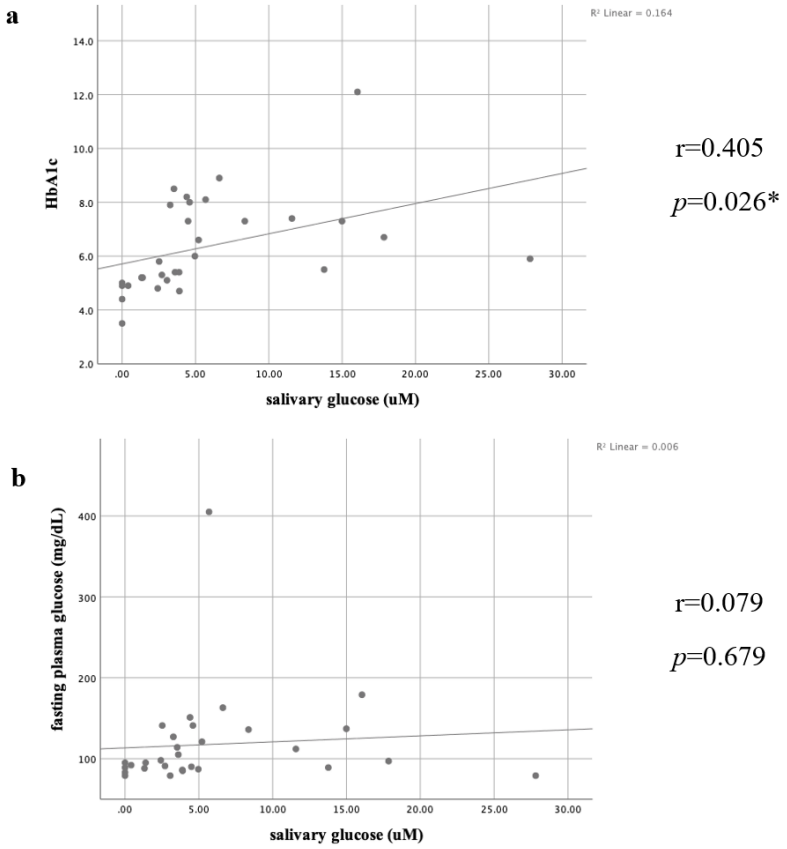
Discussion

Nowadays, the assessment of glycemic status is based on the measurement of blood glucose levels via blood sampling which is an

Table 1. Characteristics and salivary glucose levels of subjects in the control and DM groups.

Variables	All (n=30)	Control Group (n=15)	DM Group (n=15)	*p*-value
Age (years)	43.00 (29.00, 57.00)	29.00 (29.00, 43.00)	57.00 (43.00, 61.00)	0.000*
Sex (female)	20 (66.70%)	11 (73.33%)	9 (60.0%)	0.446
BMI (kg/m^2)	24.59 (20.64, 31.66)	20.86 (18.75, 23.52)	31.64 (24.62, 35.30)	0.000*
FPG (mg/dL)	96.0 (87.75, 136.25)	89.00 (85.00, 95.00)	136.00 (112.00, 151.00)	0.000*
HbA_{1C} (%)	5.85 (5.08, 7.53)	5.10 (4.80, 5.40)	7.40 (6.70, 8.20)	0.000*
Salivary glucose (µM)	3.89 (2.17, 7.06)	2.43 (0, 3.88)	5.69 (4.40, 15.00)	0.000*

Values are presented as median (1st, 3rd quartile).
* Statistically significant difference between the control and DM groups ($p<0.05$).



r: Pearson correlation coefficient.
*Statistically significant correlation (p <0.05).

Figure 1. Correlation between salivary glucose, HbA_{1C}, and fasting plasma glucose levels.
a Salivary glucose and HbA_{1C},
b Salivary glucose and fasting plasma glucose (FPG).

invasive technique. If saliva sample could be used as an alternative way to evaluate glycemic status, it would be beneficial for medical field. This cross-sectional study could reveal the potential of using salivary glucose as a biomarker of blood glucose level.

Significantly higher salivary glucose levels were revealed in diabetic subjects compared to healthy individuals. The level of salivary glucose was significantly related with HbA_{1C} which reflects overall glycemic status of subjects in a 3-month period. This finding is in line with Gupta et al. who studied the relationship between blood glucose and salivary glucose in 100 healthy subjects and 100 type 2 diabetic subjects.[8] They collected unstimulated whole fasting saliva and measured salivary glucose by using glucose peroxidase method. The fasting plasma glucose and HbA_{1C} levels were randomly determined in 40 diabetic subjects. The results showed that salivary glucose positively correlated with HbA_{1C} in the diabetic group.[8] Abikshyeet et al. studied the role of saliva whether it could be used as a diagnostic tool for DM. They recruited 106 type 2 diabetic patients and 15 healthy controls, measured HbA_{1C} levels and salivary glucose levels. The result showed that salivary glucose levels were positively correlated with HbA_{1C}.[9] Recent systematic reviews and meta-analyses showed that salivary glucose levels were elevated in type 2 DM patients and the levels were correlated with HbA_{1C}. In addition, the correlation was more obvious in subjects with high percentage of HbA_{1C}.[12] These results suggest the trend to use salivary glucose as a biomarker for type 2 DM.

The present study only revealed a non-significant correlation between salivary glucose and FPG levels. This was contradicted to other studies. Nadaf et al. assessed the correlation of FPG and fasting salivary glucose levels in 30 diabetic and 30 non-diabetic subjects.[10] They collected blood and saliva samples after 8 hours of fasting period. Salivary glucose levels were determined by hexokinase method. They showed a correlation between the levels of FPG and fasting salivary glucose.[10] Enrique et al. studied blood glucose and salivary glucose levels in 3 groups of subjects including 25 subjects with normal fasting glucose, 25 subjects with impaired fasting glucose, and 25 subjects with provisional DM. Salivary glucose levels were analyzed by using glucose oxidase-peroxidase method. The results revealed that salivary glucose levels were correlated with FPG.[11] Azizi et. al conducted an experiment in healthy controls and type 2 diabetic patients by measuring salivary glucose and FPG levels. They reported statistically significant correlation between salivary glucose and FPG levels only in the DM group but not in the healthy group and suggested that salivary glucose should not be used as a substitute for the blood glucose measurement.[17] These contradicted results may be due to the difference in the sensitivity of the test kits and the different study population.

In the present study, salivary glucose could be detected in all diabetic subjects but not in some healthy individuals (4 out of 15). This may be due to the very low level of salivary glucose in some healthy individuals. Thus, the salivary glucose may not be appropriate to be used as a biomarker in normal healthy individuals.

Collectively, salivary glucose tended to be a biomarker for blood glucose level in diabetic patients whose blood glucose was high enough to be detected in saliva. However, this study was a cross-sectional study, no causal relationship could be drawn. Further investigation in a cohort study design should be encouraged. In

addition, a greater number of subjects with age and sex matched should be recruited to confirm the result.

Conclusion

Salivary glucose levels were correlated with HbA_{1C} and had a potential to be used as biomarker for glycemic status in Thai type 2 diabetics.

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OM-00004

Efficacy of Cannabidiol for Treating Recurrent Aphthous Ulcers

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ABSTRACT

Background and Objective: Recurrent aphthous ulcer (RAU) is the most common painful oral lesion. Although topical steroids effectively treat RAU, long-term use can cause candidiasis. Cannabidiol (CBD) may be an alternative treatment for RAU due to its anti-inflammatory and wound healing-promoting effects. The objective of this study was to compare the efficacy among 0.1% CBD, 0.1% triamcinolone acetonide (TA) and placebo in the reduction of RAU size.

Methods: A total of 69 RAU subjects randomly received one of three topical interventions: 0.1% CBD, 0.1% TA or placebo. These were applied to the ulcers 3 times/day for 7 days. The pseudomembranous ulcer and erythematous border size were measured using a calibrated dental probe with millimeter markings on day 0, 2, 5 and 7.

Results: No subjects experienced adverse reactions or side effects to CBD. CBD significantly reduced the pseudomembranous ulcer size more than placebo only on day 7, while the pseudomembranous ulcer size reduction was significantly higher in the TA intervention than the placebo intervention at all evaluated time points. TA significantly reduced the erythematous border size more than placebo on day 5 and 7. However, the differences in erythematous border size reduction between subjects receiving CBD and placebo were not major.

Conclusion: Topical 0.1% CBD reduced ulcer size and accelerated ulcer healing in the late stage of RAU without any side effects. Therefore, topical CBD is perhaps more suitable for RAU patients who desire to avoid the use of topical steroids, except for cases where CBD is contraindicated.

Keywords: Cannabidiol, Cannabinoids, Cannabis, Recurrent aphthous stomatitis, Recurrent aphthous ulcer

Introduction

Recurrent aphthous ulcer (RAU) is the most common painful oral lesion and affects patient quality of life including nutrition, speech and oral hygiene.[1] RAU is a self-limiting ulceration.[2] It is more common in female sex from childhood through adolescence. Despite having a high prevalence (approximately 20% of the general population),[1] the precise etiology remains unclear.[3] However, RAU has been found associated with immunological disorders.[4] Other predisposing factors include trauma, nutritional deficiencies, food allergies, genetics, stress, microbial factors, hormonal defects and underlying medical diseases (*e.g.,* Crohn's disease, Behcet's syndrome, anemia).[1,5]

Clinically, RAU presents as an ovoid or round well-defined ulcer, with a pseudomembranous yellowish gray center and an erythematous circumscribed border.[6] RAU has been divided into three subtypes related to the number, size of ulcer and healing pattern: minor aphthous ulcer, major aphthous ulcer and herpetiform ulcer. Minor aphthous ulcer is the most common type, affecting 80% of RAU patients. It typically presents as less than 1 cm in diameter[5] and spontaneously resolves in 4-14 days without scarring.[7]

Currently, there are no curative treatments for RAU. Hence, the RAU management primarily focuses on pain relief, reducing inflammation and promoting wound healing to reduce the number, duration and size of ulcer. Although topical steroids are an effective first-line medication for RAU,[8,9] they also have numerous side effects, especially suppressing the immune response that can lead to developing oral candidiasis from long-term steroid use.[10] Natural substances such as Damask rose and quercetin have been supported as alternative treatments for RAU due to their anti-inflammatory and wound healing-promoting properties respectively.[9]

In recent years, there is considerable public interest in the use of cannabis for medical purposes. One of the major medicinal components of cannabis are cannabinoids. They are synthesized in the human body called endocannabinoids and produced by the cannabis plant called phytocannabinoids. The two active medical components from the cannabis plant are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD).[11] THC has several medicinal effects including being psychoactive. In contrast, CBD is non-psychoactive and has meaningful analgesic, anti-inflammatory and anti-convulsant effects.[12] Furthermore, CBD suppresses pro-inflammatory cytokine expression.[13,14] Inflammation plays a critical role in the wound healing process.[15] However, excessive inflammatory mediators can delay healing.[16,17] Hence, reducing pro-inflammatory cytokine expression optimizes healing time and reduces pain intensity.[17] Thus, CBD may promote wound healing due to its anti-inflammatory properties.

European Medicinal Cannabis Association (EUMCA) regulated that products from cannabis extracts must have CBD as the main component in high purity and limit no more than 0.2% by weight of THC to reduce and prevent misuse of cannabis extracts and be considered for medicinal purposes.[18] Medicinal cannabis products are defined as dried female flowers of *Cannabis sativa* or *Cannabis indica* whose concentration levels range from 0.1% to 19% for CBD.[19] Cannabis side effects are dose dependent. Most adverse events of

cannabis can be mitigated by using a 'start low and go slow' dosing strategy.[20] Recently, there is a study that assessed the safety and preventive effects of 0.1% CBD on chemotherapy-induced peripheral neuropathy (CIPN) in patients receiving oxaliplatin- or paclitaxel-based chemotherapy which concluded that CBD rarefied early symptoms of CIPN without major adverse events.[21]

The safety of CBD has been demonstrated in animal and human studies. It has a low adverse effect profile, even when chronically used.[22] However, there are some reports of sedation and somnolence with CBD use. Prescribing CBD with other sedative drugs like opioids and benzodiazepines can cause severe respiratory depression. Contraindications to CBD administration include allergies to cannabidiol (signs of cutaneous irritation or anaphylactic reaction), history of drug or alcohol addiction (due to CBD addictive properties) and history of mood disorders, depression or suicidal thoughts (due to correlation with increased suicidal thoughts and behavior of CBD use).[23]

In summary, CBD extracted from cannabis can perhaps be an alternative treatment for RAU due to its known clinical benefits, particularly in reducing inflammation and promoting wound healing.[24] In the dental area, we found a study using CBD in oral traumatic ulcerative lesions in rats, which determined that CBD reduced inflammation in the early stage of the wound healing process in microscopic findings although it did not promote clinical improvement adequately.[25] Recently, a study demonstrated that topical application of CBD on trauma- and acid-induced ulcers on mice tongues relieved pain, inhibited inflammation and promoted wound healing.[24] Yet, topical 0.1% CBD has not been assessed for the clinical safety and efficacy in the treatment of RAU.

The objective of this study was to compare the efficacy among 0.1% CBD, 0.1% triamcinolone acetonide (TA) and placebo (pure oral paste) in the reduction of RAU size.

Materials and Methods

CBD Preparation

Cannabis sativa was acquired from a local herbal supplier in Bangkok, Thailand. CBD was obtained by supercritical carbon dioxide extraction using ethanol as the co-solvent and separated by centrifugal partition chromatography.[26] Purification was performed using centrifugal partition chromatography.[27] The cannabinoid composition (CBD content) of the obtained extracts was determined by high performance liquid chromatography analysis.[28] CBD oral pastes were prepared by the Faculty of Pharmaceutical Sciences, Chulalongkorn University (Bangkok, Thailand) and passed cellular and animal safety screening (see Additional file 1).

Population and Sample

Sample Population

The RAU patients from the Oral Medicine Clinic at the Faculty of Dentistry, Chulalongkorn University, who met the inclusion and exclusion criteria.

Sample Size Calculation

The sample size was calculated by using the G*Power program version 3.1.9.7 for 80% power and 95% confidence interval level according to the range of ulcer sizes reported in a previous study.[29] The final estimated total sample size was 60. To compensate for error or loss of subjects during follow-up, a total sample size of 69 subjects were recruited.

Inclusion and Exclusion Criteria

Inclusion Criteria

1. Age between 18-65 years old.
2. Willing to participate and provide informed consent.
3. Having a history of RAU (at least 2 times/year) on the nonkeratinized oral mucosa.
4. Presenting with 1-3 minor aphthous ulcers that were 2-10 mm in diameter and ≤48 hours duration.
5. Ulcer sites were easily accessible for treatment and evaluation (*e.g.,* buccal mucosa, labial mucosa, floor of mouth).

Exclusion Criteria

1. History of allergies to CBD.
2. Pregnancy/lactation.
3. Concurrent oral bacterial/viral/fungal infections.
4. Ulcers as a manifestation of systemic diseases such as Behcet's syndrome, Crohn's disease, ulcerative colitis or anemia.
5. Ulcers from trauma.
6. Diabetes mellitus patients.
7. Treatment with systemic steroids, immunomodulatory agents or oral retinoids within 1 week.
8. Treatment with acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDs) or other oral topical medications within 48 hours or during study participation.
9. History of dental surgery within 2 weeks of entering the study.
10. Orthodontic braces or retainers that might come in contact with the ulcers.

Ethics Approval

The present study was performed with informed consent following protocols approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (certificate number HREC-DCU 2021-048 approved on 9 July 2021).

Study Design

To measure the efficacy of CBD for treating RAU, 69 subjects with RAUs randomly received one of three topical interventions: 0.1% CBD, 0.1% TA or placebo. This study was a randomized parallel double-blind controlled clinical trial. A research assistant generated the random allocation sequence, enrolled subjects and assigned subjects to interventions. Subjects and investigator were both blinded to the type of intervention. The interventions were applied with a provided calibrated spoon to the ulcers 3 times/day after meals for 7 days.

The ulcer size was measured on day 0, 2, 5 and 7. Two ulcer size parameters consisting of the pseudomembranous ulcer and erythematous border size were measured. The ulcer diameters were measured using a calibrated dental probe with millimeter markings and the ulcer sizes were calculated using formulas for the surface area of a circle or ellipse. In case of a circle area, we measured a diameter. If ulcers were an ellipse, we measured the longest and the shortest diameters, then calculated the area of the ulcer with these formulas:

Area of a circle in mm^2 (A) = πr^2

π = 22/7

r = radius of a circle or diameter/2 (mm)

Area of an ellipse in mm^2 (A) = πab

π = 22/7

a = the longest radius of an ellipse or the longest diameter/2 (mm)

b = the shortest radius of an ellipse or the shortest diameter/2 (mm)

If there was more than one ulcer, the ulcer with the easiest access was selected for investigation. When subjects developed RAU more than one time (at least 2 weeks apart), they could reenter the study, and received a different topical intervention. Each subject was interviewed at each visit by the same investigator regarding the emergence of any adverse reactions.

Statistical Analysis

The background and demographic data were summarized using descriptive statistics. The normal distribution for each variable was determined by the Kolmogorov-Smirnov test. Group differences among the three interventions were compared using the one-way ANOVA followed by the Bonferroni post hoc test (normally distributed variables) or the Kruskal-Wallis test followed by the Bonferroni correction for multiple tests (not normally distributed variables) for pseudomembranous ulcer and erythematous border size at each monitoring point. The data were analyzed using the SPSS software (SPSS 28 for Windows; SPSS, Chicago, IL, USA). A *p*-value of ≤0.05 was considered significant.

Results

The 69 RAU subjects (23 subjects in the CBD group, 22 subjects in the TA group and 24 subjects in the placebo group) were enrolled in this study. The normality tests of demographic data and ulcer histories determined by the Kolmogorov-Smirnov test indicated that all baseline variables were not normally distributed (p<0.001). There were no significant differences between the three interventions regarding their demographics and ulcer histories (Table 1), except for the ulcer duration (p=0.039). However, after adjusting the data with the Bonferroni correction for multiple tests, the statistical difference was no longer significant.

Table 1. Demographic data of all study participants.

Characteristic	CBD (n=23)	TA (n=22)	Placebo (n=24)	*p*-value
Age (years), mean (SD)	36.74 (11.32)	35.91 (10.38)	33.00 (10.11)	0.410[a]
Sex				
Male, n (%)	2 (2.90)	4 (5.80)	7 (10.14)	0.199[b]
Female, n (%)	21 (30.43)	18 (26.09)	17 (24.64)	
Duration of the ulcer (hours), mean (SD)	42.78 (10.51)	35.05 (13.59)	34.67 (12.96)	0.039[a,c]
Pseudomembranous ulcer size on day 0 (mm^2), mean (SD)	5.92 (4.20)	6.77 (3.97)	6.74 (5.18)	0.530[a]
Erythematous border size on day 0 (mm^2), mean (SD)	13.79 (9.28)	14.70 (9.37)	17.38 (17.47)	0.604[a]

[a]From Kruskal-Wallis test.
[b]From Pearson's chi-square test.
[c]After adjusting the data with the Bonferroni correction for multiple tests, the statistical difference was no longer significant (TA-placebo: p=1.000, CBD-placebo: p=0.070 and TA-CBD: p=0.099).

CBD Safety Clinical Outcomes after Application on the Ulcers

No subjects experienced obvious adverse reactions (irritant response or allergic reaction) in the oral mucosa. Also, no signs of liver dysfunction were noticed (such as nausea, vomiting, jaundice, right upper quadrant pain or dark urine) when applied CBD to the ulcers for 7 days.

Pseudomembranous Ulcer Size Reduction

The pseudomembranous ulcer size was adjusted to a percentage compared with baseline (100%) as demonstrated in Table 2. The normal distribution of adjusted percentage pseudomembranous ulcer size at each monitoring point determined by the Kolmogorov-Smirnov test is summarized in Table 3.

The pseudomembranous ulcer size reduction analysis among the three interventions indicated that the pseudomembranous ulcer size was approximately 50% smaller in the CBD group on day 2 compared with the placebo group as shown in Fig. 1. The average pseudomembranous ulcer size in the placebo group on day 2 increased approximately 155% compared with baseline. TA reduced the pseudomembranous ulcer size from day 2 onwards, while CBD slightly increased the pseudomembranous ulcer size on day 2 and then decreased the pseudomembranous ulcer size from day 5 onwards. In contrast, the placebo markedly increased the pseudomembranous ulcer size on day 2 and 5, however, this size was decreased on day 7.

Statistical analysis revealed that CBD significantly reduced the pseudomembranous ulcer size more than placebo only on day 7 (p=0.033) as demonstrated in Table 4, while the pseudomembranous ulcer size reduction was significantly higher in the TA group compared with the placebo group at all evaluated time points (p<0.05). Although CBD reduced the pseudomembranous ulcer size less than TA, the differences were not major.

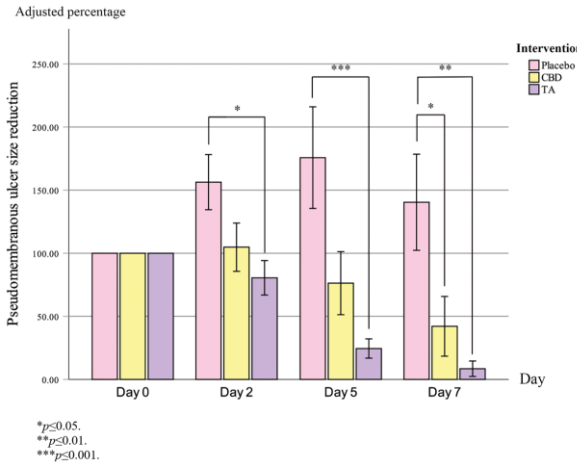

Figure 1. Pseudomembranous ulcer size reduction. Y-axis values represent percentages. Error bars display the standard error of the mean (SEM).

Erythematous Border Size Reduction

The erythematous border size was adjusted to a percentage compared with baseline (100%) as presented in Table 2. The normal distribution of adjusted percentage erythematous border size at each monitoring point determined by the Kolmogorov-Smirnov test is summarized in Table 3.

The erythematous border size was 50% smaller in the CBD group on day 7 compared with the placebo group as demonstrated in Fig. 2. The average erythematous border size in the placebo group on day 7 increased approximately 125% compared with baseline. TA reduced the erythematous border size from day 2 onwards, while CBD slightly increased the erythematous border size on day 2 and then decreased the erythematous border size from day 5 onwards. In contrast, the placebo markedly increased the erythematous border size on day 2 and 5, however, this size was decreased on day 7.

Table 2. Comparison of adjusted percentage pseudomembranous ulcer and erythematous border size at each monitoring point compared with baseline (100%), mean (SD).

Variable	CBD (n=23)	TA (n=22)	Placebo (n=24)	*p*-value
Pseudomembranous ulcer size				
Day 0	100.00 (0.00)	100.00 (0.00)	100.00 (0.00)	
Day 2	104.77 (91.59)	80.53 (64.05)	156.32 (106.96)	0.024[a]
Day 5	76.27 (119.90)	24.48 (35.88)	175.76 (197.28)	0.002[a]
Day 7	42.05 (113.43)	8.47 (28.61)	140.39 (186.67)	0.001[a]
Erythematous border size				
Day 0	100.00 (0.00)	100.00 (0.00)	100.00 (0.00)	
Day 2	108.95 (52.42)	87.14 (57.55)	126.38 (56.73)	0.065[b]
Day 5	98.89 (97.11)	49.86 (59.36)	132.88 (94.01)	0.003[a]
Day 7	73.92 (121.10)	26.80 (46.71)	123.46 (129.27)	0.004[a]

[a]From Kruskal-Wallis test.
[b]From one-way ANOVA.

Table 3. Normality tests of adjusted percentage pseudomembranous ulcer and erythematous border size at each monitoring point determined by the Kolmogorov-Smirnov test.

Variable	*p*-value
Pseudomembranous ulcer size	
Day 2	<0.001
Day 5	<0.001
Day 7	<0.001
Erythematous border size	
Day 2	0.200
Day 5	<0.001
Day 7	<0.001

Statistical analysis revealed that TA significantly reduced the erythematous border size greater than placebo on day 5 (p=0.002) and day 7 (p=0.003) as presented in Table 4. However, the differences in erythematous border size reduction between subjects receiving CBD and placebo were not major. Although CBD reduced the erythematous border size less than TA, the differences were not significant.

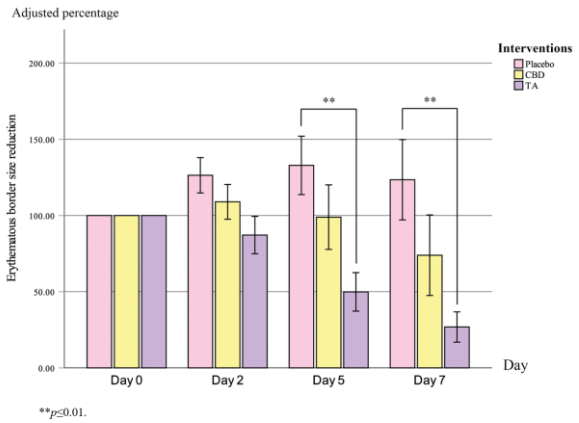

Figure 2. Erythematous border size reduction. Y-axis values represent percentages. Error bars display the SEM.

Table 4. Multiple comparisons of adjusted percentage pseudomembranous ulcer and erythematous border size at each monitoring point among the three interventions.

Variable	TA-Placebo	CBD-Placebo	TA-CBD
Pseudomembranous ulcer size			
Day 2	0.023[a]	0.214[a]	1.000[a]
Day 5	0.001[a]	0.097[a]	0.448[a]
Day 7	0.002[a]	0.033[a]	1.000[a]
Erythematous border size			
Day 2	0.060[b]	0.897[b]	0.612[b]
Day 5	0.002[a]	0.509[a]	0.151[a]
Day 7	0.003[a]	0.394[a]	0.247[a]

[a]From Kruskal-Wallis test after adjusting significant values with the Bonferroni correction for multiple tests.
[b]From one-way ANOVA followed by the Bonferroni post hoc test.

Discussion

The present study evaluated the use of 0.1% CBD as a topical treatment for RAU. No subjects experienced adverse reactions or side effects to 0.1% CBD when applied to the ulcers for 7 days. Therefore, topical 0.1% CBD appears to be safe for oral mucosa applications.

The roles of CBD in oral lesions are pain relief, inhibiting inflammation and promoting wound healing.[24] The previous studies had only focused on *in vivo* CBD effects in oral traumatic ulcerative lesions,[24,25] however, a clinical trial of RAU is lacking. Pseudomembranous ulcer and erythematous border size were evaluated in this study. These are standard variables that are often assessed when evaluating the pharmacological efficacy for RAU management. The findings from the present randomized, double-blind controlled clinical trial study indicate that CBD treatment reduced the pseudomembranous ulcer size in the late stage of RAU during the 7-day application. The ulcer size was significantly reduced due to the wound healing promotion and anti-inflammatory effects of CBD similar to a study evaluating CBD effects, which demonstrated that topical application of CBD could promote wound healing and inhibit inflammation on trauma- and acid-induced ulcers on mice tongues.[24] The erythematous circumscribed border represents the level of inflammation.[30] Although CBD reduced the erythematous border size greater than placebo, the differences did not reach statistical significance. Similar to a study using CBD in oral ulcerative lesions in rats, which determined that CBD exerted an anti-inflammatory effect in the early phase of wound healing in microscopic findings although it did not promote clinical improvement adequately.[25]

This study has its own limitations that must be carefully considered. One of the limitations of the present study was the accuracy of ulcer size measurement. In this study, we measured the ulcer size with a calibrated dental probe with millimeter markings. Although the ulcer diameters measured using a dental probe were calculated using formulas for the surface area of a circle or ellipse, the exact ulcer size is quite difficult to calculate due to the imperfect round or ovoid shape ulcers and could have resulted in inaccurate ulcer sizes. To minimize this inaccuracy, analysis of ulcer size by using computer software would be more precise for assessing improvement rates during RAU treatment interventions. Furthermore, ulcer size reduction is not the only sign of improvement in healing ulcers, decreases in pain intensity and ulcer depth, satisfaction and quality of life improvement should also be measured. If these can be incorporated into the ulcer healing outcomes, the findings may be more comprehensive.

To our knowledge, this is the first randomized clinical trial investigating the clinical effects of 0.1% CBD for RAU topical treatment. The efficacy of CBD was clinically meaningful specifically on the reduction of the pseudomembranous ulcer size in the late stage of RAU.

Conclusion

This clinical study demonstrated that topical 0.1% CBD reduced ulcer size and accelerated ulcer healing in the late stage of RAU without any reported side effects. Hence, CBD is perhaps more suitable for RAU patients who decline to take topical steroids, except for those cases where CBD is contraindicated such as in the presence of allergies to CBD, history of drug or alcohol addiction and history of mood disorders.

Acknowledgements

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OM-00005

Urinary and Water Fluoride Concentrations in Dental Fluorosis Patients in Thailand

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ABSTRACT

Background and Objective: Excessive fluoride intake during tooth development may result in dental fluorosis which is one of the most common signs of fluoride exposure in humans. Water fluoride concentration is a common indicator for fluoride measurement in a national survey. Currently, there is no updated studies on urinary and water fluoride concentrations in dental fluorosis patients in Thailand. This study aimed to assess severity of dental fluorosis, fluoride content in urine and drinking-water of schoolchildren in fluoride endemic areas.

Methods: Schoolchildren in fluoride endemic areas which located in Lamphun and Ratchaburi provinces, Thailand were screen for fluorosis severity (n=50). The 24-hours urine and drinking-water were collected. The fluoride concentrations in urine and drinking-water were evaluated by ion-selective fluoride electrode.

Results: Among 50 schoolchildren aged between 6-16 years; 30 were non-dental fluorosis and 20 were moderate to severe (M-S) dental fluorosis. Urine fluoride concentrations in M-S group were significantly higher than those in non-fluorosis group while water fluoride concentrations in M-S group were not significantly different from non-fluorosis group. A positive correlation (r=0.49) between urine and water fluoride concentrations (p<0.001) was observed. The living duration of the parents in fluoride endemic areas was significantly correlated with urinary fluoride concentration of children.

Conclusion: The study shows that the children who have moderate-severe fluorosis have increased urinary fluoride concentration. Apart from drinking-water, other sources such as foods and dental products can contribute to urinary fluoride concentration and development of dental fluorosis.

Keywords: Dental fluorosis, Fluoride endemic areas, Urinary fluoride excretion, Water fluoride concentration

Introduction

Fluoride is found in water, food and especially dental products. It is considered as a double-edged sword. Fluoride provides a protection against dental caries when consuming in adequate quantity, but chronic and excessive fluoride intake can cause dental and skeletal fluorosis.[1]

Dental fluorosis is a developmental disturbance of dental enamel, caused by chronic and excessive ingestion of fluoride during tooth development.[2] In Thailand, the national standard of fluoride concentration in drinking water is ≤ 0.7 mg/liter and not exceed 1.0 mg/liter during high water consumption in summer. Dental fluorosis is endemic among certain areas depending on geological background such as the northern and western parts of Thailand. A high incidence of dental and skeletal fluorosis has been reported in Chiangmai, Lamphun and Phayao provinces due to high fluoride concentration in natural water sources.[3] It has been estimated that fluoride concentration in underground water at endemic area can reach 10 mg/L(ppm).[4]

The common source of fluoride exposure is the drinking/cooking water derived from underground water. Fluoride in water cannot be removed by boiling or distillation. Reverse osmosis is the successful defluoridation process in community scale, but it comes with high investment and maintenance cost.[5] After ingestion within minutes, fluoride is rapidly absorbed from the stomach to plasma. The peak plasma fluoride level usually occurs within the next 30-60 minutes, and then declines due to bone uptake and urinary excretion. Urinary fluoride is a widely accepted biomarker of current fluoride exposure and has frequently been used as an indicator of fluoride exposure from drinking-water recommended by WHO.[6]

Dean's fluorosis index (DFI) is often used by the World Health Organization and remains as the standard gold index in public health armamentarium. It consists of 6 categories based on the second most severely affected clinical appearance. The normal teeth are given a score of 0. The score gradually increases by the severity to score of 4 which stands for the most severe type of fluorosis.[7]

This aims of this study were to assess severity of dental fluorosis, and fluoride content in urine and drinking-water in schoolchildren in fluoride endemic areas of Thailand.

Materials and Methods

The protocol for this study was approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand (Study Code HREC-DCU 2021-061, approved on 1 October 2021)

Sample Size Calculation

The sample size was calculated by using the n4Studies program version 1.4.1 for 80% power and 95% confidence interval

level according to the previous study.[8] The final estimated total sample size was 50.

Screening and Selection of Subjects

Participant screenings were held in fluoride endemic area of Thailand located in Lamphun and Ratchaburi provinces according to their high prevalence of dental fluorosis and low population mobility.

The inclusion criteria of case group were Thai population with moderate to severe dental fluorosis (Dean fluorosis score 3-4) and those of the control group were Thai population without dental fluorosis (Dean fluorosis score 0). DFI and diagnostic criteria are shown in Table 1. A consensus score for DFI was given for each subject by four examiners (P.G., T.P., P.A and a pediatric dentist). The final score for DFI was recorded directly by the interface excel file.

All subject recruited must not have the following conditions: clinical dental caries, systemic condition, history of antibiotic, antifungal, antiviral or steroid intake or use of mouthwash in 3 months prior to sample, presence of periodontitis and mucosal lesions, smoking, drinking alcohol, and using narcotic drugs.

Informed consents were obtained from parents and/or child participants (ages 6 to 16 years) included in this study.

History Taking and Questionnaire

The questionnaire including information of age, gender, grade, period of staying in the area and medical history of the participants and their parents were collected.

Sample Collection and Fluoride Measurement

All subjects were asked to collect their 24-hr urine in 2.4 L container and the most consumption drinking-water in household in 60 ml. bottle. The samples were transported to the laboratory at Intercountry Centre for Oral Health, Chiangmai for fluoride measurement. The amount of fluoride was evaluated by TISAB III, containing CDTA (cyclohexylenedinitrilotetraacetate), sodium hydroxide, sodium chloride and acetic acid (ethanoic acid) dissolved in deionized water. Fluoride electrode (4-star benchtop, Orion, USA) was used as the measuring equipment.

Statistical Analysis

Statistical analysis was performed using SPSS software version 22 (SPSS Inc. Chicago, IL, USA). Demographic and clinical conditions were reported with descriptive statistics. The normal distribution for each variable was determined by the Kolmogorov-Smirnov test and follow by nonparametric Mann-Whitney U test. Statistical significance level of less than 0.05 was considered significant.

Results

Seventy-one subjects from 3 schools in endemic area were approached to participated in this study. Twenty-one subjects did not provide consent to participate or fail to collect samples. Fifty subjects were included. Oral examination and sample collection took place between December 2021 to January 2022.

Subjects were male 44% and female 56%, mean age 11.26 ± 2.76 years. The children were divided into 2 groups; control group (n=30) who were non-dental fluorosis (Dean fluorosis score 0); male 46.7% and female 53.3% and case group (n=20) who were moderate to severe dental fluorosis (Dean fluorosis score 3-4); male 40% and female 60%.

Mean fluoride value in total water samples were measured to be 1.52 (range from 0.10 to 16 ppm) and mean fluoride value in total urine sample of children were found to be 3.20 (range from 0.10 to

Table 1. Criteria for Dean's fluorosis index for dental fluorosis.

Classification	Score	Criteria
Normal	0	The enamel represents the usual translucent semivitriform (glass-like) type of structure. The surface is smooth, glossy, and usually of a pale, creamy white color.
Questionable	0.5	The enamel discloses slight aberrations from the translucency of normal enamel, ranging from a few white flakes to occasional white spots. This classification is used in those instances where a definite diagnosis of the mildest form of fluorosis is not warranted and classification of "normal" not justified.
Very Mild	1	Small, opaque, paper white areas scattered irregularly over the tooth, but not involving as much as approximately 25 percent of the tooth surface. Frequently included in this classification are teeth showing no more than about 1-2 mm of white opacity at the tip of the summit of the cusps of the bicuspids or second molars.
Mild	2	The white opaque areas in the enamel of the teeth are more extensive, but do not involve as much as 50 percent of the tooth.
Moderate	3	All enamel surfaces of the teeth are affected, and surfaces subject to attrition show wear. Brown stain is frequently a disfiguring feature.
Severe	4	All enamel surfaces are affected and hypoplasia is so marked that the general form of the tooth may be affected. The major diagnostic sign of this classification is discrete or confluent pitting. Brown stains are widespread, and teeth often present a corroded-like appearance.

11.40 ppm). In control group, mean fluoride value of urine sample was 2.43 ppm (range from 0.10 to 6.78 ppm) and mean fluoride value of water sample was 1.40 ppm (range from 0.10 to 16 ppm) while in case group, mean fluoride value of urine sample was 4.35 (range from 0.70 to 11.40) and was found to be 1.71 (range from 0.10 to 7.50 ppm) in water sample. There was not significantly different between case and control group in parent's duration of living characteristic (p=0.13). The result showed that in case group, the parent of participant also experienced tooth discoloration (50%) and others (50%) had no report of any fluoride related diseases while in control group none of those were experienced tooth discoloration but 20% experienced joint problem and other 80% had no report of any fluoride related diseases. General characteristics of the study population are shown in Table 2.

Analyzing the urinary and water fluoride concentrations between M-S group and non-fluorosis group showed that urine fluoride concentrations in fluorosis group were significantly higher than those in non-fluorosis group (p<0.05) while the water fluoride concentrations in fluorosis group was not significantly different from non-fluorosis group (p=0.30) (Fig. 1).

A significantly positive correlation was observed between urinary fluoride and water fluoride (r=0.49, p<0.05) as shown in Fig. 2.

In relation to the presence of fluoride in urine and water samples of children and parent's duration of living in endemic area,

Table 2. General characteristics of the study population.

Characteristics	Total (N=50)	Control (N=30)	Case (N=20)	*p*-value
Age (years)				0.005
○ Mean ± SD	11.26 ± 2.76	12.13 ± 2.80	9.95 ± 2.00	
○ Median	11	12.50	11	
○ Range	6 to 16	6-16	6-12	
Sex				0.645
○ Male	22 (44%)	14 (46.7%)	8 (40%)	
○ Female	28 (56%)	16 (53.3%)	12 (60%)	
Dean Index				
○ Score 0	34 (60%)	30 (100%)	0	
○ Score 3	8 (16%)	0	8 (40%)	
○ Score 4	12 (24%)	0	12 (60%)	
Urine Fluoride (ppm)				0.005
○ Mean ± SD	3.20 ± 2.52	2.43 ±1.99	4.35 ± 2.83	
○ Median	2.78	1.81	3.41	
○ Minimum	<0.10	<0.10	0.70	
○ Maximum	11.40	6.78	11.40	
Water Fluoride (ppm)				0.30
○ Mean ± SD	1.52 ± 2.97	1.40 ±3.26	1.71 ± 2.54	
○ Median	0.20	0.20	0.22	
○ Minimum	<0.10	<0.10	<0.10	
○ Maximum	16.00	16.00	7.50	
Location				0.05
○ Lamphun	26 (52%)	19 (63.3%)	7 (35%)	
○ Ratchaburi	24 (48%)	11 (36.7%)	13 (65%)	
Parent's Duration of Living				0.13
○ 1-5 years	0	0	0	
○ 5-10 years	24 (48%)	18 (60%)	6 (30%)	
○ 10-20 years	4 (8%)	2 (6.7%)	2 (10%)	
○ More than 20 years	22 (44%)	10 (33.3%)	12 (60%)	
Parent History				0.004
○ None	34 (68%)	24 (80%)	10 (50%)	
○ Bone Fracture	1 (1.4%)	0	0	
○ Joint Pain	6 (12%)	6 (20%)	0	
○ Tooth Discolor	10 (20%)	0	10 (50%)	

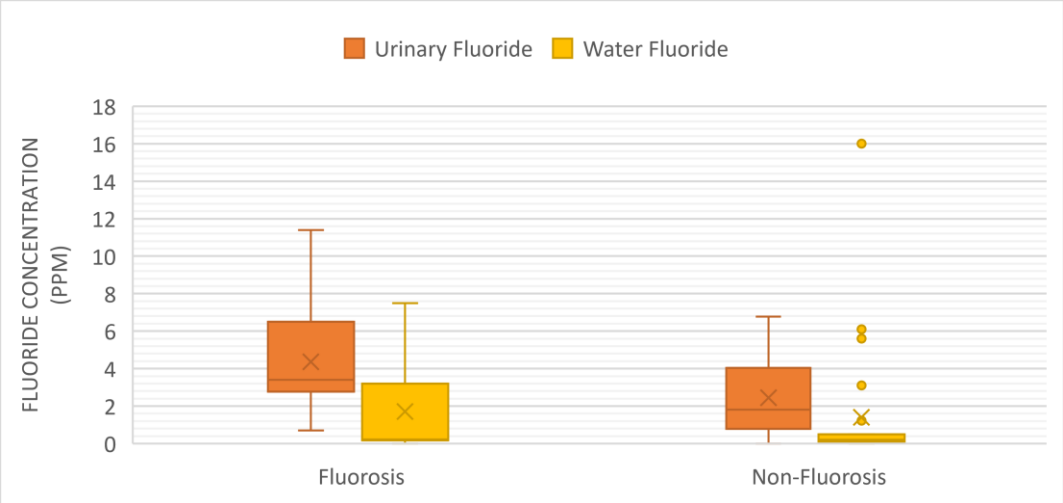

Figure 1. The fluoride concentration (ppm) of urine and water samples between dental fluorosis group and non-dental fluorosis group.

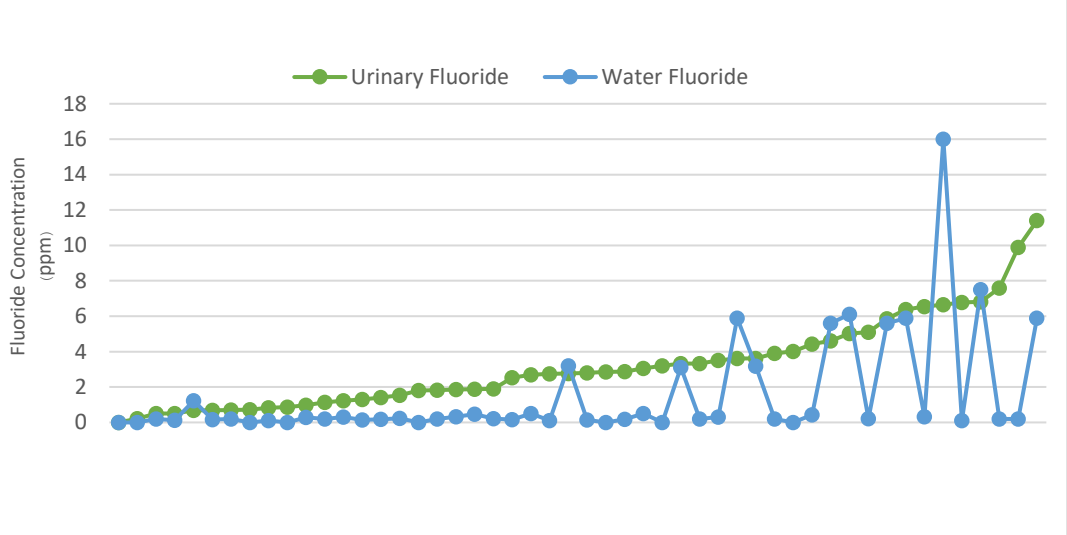

Figure 2. The association between fluoride concentration in water sample and the urinary fluoride concentration for each participant, the data is ordered according to urinary concentration.

Table 3 and Fig. 3 show the mean fluoride urinary value of 2.39 ±1.91 ppm in group 5-10 years of parents' living duration in endemic area (n=24), 3.63 ± 2.3 ppm in group 10-20 years of living duration (n=4), and 4.01 ± 2.93 ppm in group more than 20 years of living duration (n=22). A significant positive moderate strength correlation (r=0.23, p<0.05) between the parent's duration of living and urine fluoride concentration was observed while water fluoride concentration did not show a significant correlation with parent's duration of living (r=0.03, p=0.79).

Discussion

The maximum concentration of fluoride in natural water at 1.5 ppm is set as the guideline value to prevent the incidence of dental fluorosis according to "Guidelines for Drinking-Water Quality" by WHO 2006, while the recommendation for artificial fluoridation of water supply is at 0.5-1.0 ppm.[9]

Drinking water consumption depends on the atmospheric temperature. This study was carried out in hot climates region where the temperature ranged 38-40 degree Celsius. The optimal concentration of fluoride in drinking water for region with hot climates should be 0.7-1.0 ppm recommended by World Health Organization.[10] In Thailand, the national standard of fluoride concentration in drinking water was limited at 0.7 ppm. which laid down by the Bureau of Dental Health.[11] In our study, we found that the mean values of water fluoride in the endemic areas (1.52 ± 2.97 ppm) exceeded the national concentration and had a wide range of fluoride values from <0.1 to 16 ppm.

The fluoride concentrations in biomaterials such as urine and blood have been proposed as the most reliable indicators of exposure to fluoride.[12] The acceptable concentration of urine fluoride is 1 ppm.[13] In our study, the fluoride concentration in urine samples from the endemic areas was higher than the accepted level. The mean urinary fluoride was at 3.20 (range from 0.1 to 11.40 ppm). Other studies of urinary fluoride in other endemic areas showed 0.17-47.50, 0.15-1.99, 0.9-3.25, 1.07-4.0 and 0.05-2.8 ppm in Youssoufia city of Morocco,[14] Barcelona,[15] Gurgaon,[16] Nellore[13] and Jhajjar,[17] respectively.

Table 3. The relationship between parent's duration of living in fluoride endemic areas and fluoride concentrations in urine and water samples of children.

Parent's Duration of Living	N	Urine Fluoride (ppm)	Water Fluoride (ppm)
Less than 5 years	0		
○ Mean ± SD		N/A	N/A
○ Range		N/A	N/A
5-10 years	24		
○ Mean ± SD		2.39 ±1.91	1.67 ± 3.63
○ Range		<0.1 – 6.81	< 0.1 – 16.0
10-20 years	4		
○ Mean ± SD		3.63 ± 2.3	1.50±2.73
○ Range		0.7 - 5.86	<0.1 - 5.6
More than 20 years	22		
○ Mean ± SD		4.01 ± 2.93	1.36 ± 2.24
○ Range		0.68 – 11.40	<0.1 – 5.9

Epidemiological studies have showed that people who live in fluoride endemic or artificial-fluoridated area are more susceptible to dental fluorosis than those living in non-endemic/non-fluoridated area. However, many studies showed that susceptibility and severity of fluorosis is influenced by many factors, including total fluoride intake, type of fluoride intake (i.e., ingested vs. inhaled), renal function, rate of bone metabolism, metabolic activity, high altitude of residence associated with altered acid-base balance in body, nutritional status, composition of diet, and genetics.[18] These factors may contribute to the incidence and severity of dental fluorosis in individuals although they have been living in the same fluoride endemic area.

The effects of fluoride are cumulative and depend mostly, but not entirely, on the amount and duration of exposure.[19] This study supported the recent evidence that higher urinary fluoride was found in M-S dental fluorosis patients and can be useful for determining the current availability of fluoride.[20] Nevertheless, the recent biomarkers of fluoride such as hairs and nails should be determined in further study.

Our study shows a positive correlation between urinary fluoride and water fluoride concentrations although it is not linear which could be due to other sources of fluoride exposure. Apart from drinking-water which is the main source of fluoride exposure,[21] there are other fluoride sources including foods/vegetables planted in endemic areas, brick tea consumption, cooking methods and dental products.

Previous studies reported that fluoride can pass through the placenta.[22] After removal of fluoride from community waters, the half-life of fluoride loss in adults is 120 weeks and in children is 70 weeks proximately.[23] Here, we observed that the duration that the

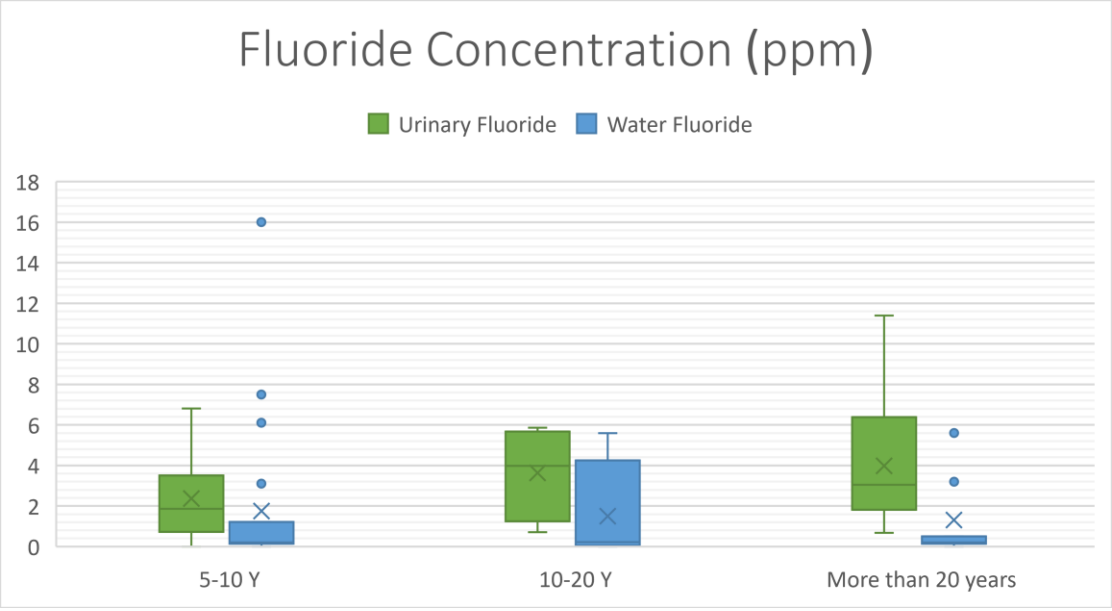

Figure 3. The fluoride concentration (ppm) in urine and water samples of children according to the durations of parents' living in fluoride endemic areas.

parents were living in endemic area was associated with the increase in children' urinary fluoride concentrations, suggesting a cumulative fluoride deposition in these children. Future studies recruiting the children who migrate from the endemic areas would clarify the above correlation.

Conclusion

This study reports the exceeding standard value of mean urinary and water fluoride concentrations in both fluorosis and non-fluorosis group in the endemic area reflecting that the schoolchildren in the areas are likely to be overly exposed to fluoride. The urinary fluoride can be used as the reliable biomarker for recent fluoride exposure other than drinking water although there is a positive correlation between urinary fluoride and water fluoride concentration. The longer duration of living in endemic area can raise the urinary fluoride concentration over the accepted level. The intervene of defluoridation and mitigation in community level should be revised and further research of fluoride toxication should be funded.

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Effect of Saliva Decontamination Methods on Bond Strength of Developed Zirconia Ceramic

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ABSTRACT

Background and Objective: Saliva-contaminated zirconia surface leads to decrease in bond strength of resin cement, an effective decontamination method will enhance bond strength. This study aimed to investigate the effect of different saliva decontamination methods on microshear bond strength (μSBS) between a Developed zirconia ceramic (DZ) and resin cement.

Methods: Forty-two zirconia plates (Cercon® xt and DZ each) were polished, treated with sandblast and divided into 6 groups; Group 1 (NC): no contamination; Group 2 (IC): Ivoclean; Group 3 (EN): 70% ethanol; Group 4 (SH): 1% sodium hypochlorite; Group 5 (SB): sandblasted with 50 micrometer (μm) aluminum oxide particles; Group 6 (W): no decontamination. Two samples were surface explored by scanning electron microscope (SEM) and X-ray photoelectron spectroscopy (XPS). Specimens were applied with CLEARFIL™ CERAMIC PRIMER PLUS and cemented with PANAVIA™ V5 by injecting into polyethylene tubes (4 rods/sample). After water storage for 24 hrs., specimens were subjected to a μSBS test at cross-head speed of 1 mm/min. Data were analyzed using Two-way ANOVA and Dunnett's T3. Modes of failure were categorized by SEM.

Results: XPS analysis showed similar element distributions between group NC, IC, SH and SB. No statistically significant difference (p>0.05) in μSBS between Cercon® xt and DZ in all group. Bond strength of group NC, IC, SH and SB were significantly higher (p<0.05) than EN and W. Failure mode analysis showed predominantly mixed failure in every group.

Conclusion: The use of Ivoclean, sodium hypochlorite and sandblasting were effective in removing saliva contamination and regaining bond strength between zirconia and resin cement.

Keywords: Bond strength, Decontamination, Resin cement, Saliva, Zirconia

Proceeding

Introduction

The translucent yttrium-stabilized zirconia (Yttria Tetragonal Zirconia Polycrystal; Y-TZP) is increasing in popularity for use as prosthetic restoration in anterior and posterior teeth due to its esthetic, strength and biocompatibility.[1-3] However, the treatment cost for zirconia restoration is still high and the treatment access was limited in some patients. National research council of Thailand in collaboration with Department of Physics and Materials science, Faculty of Science and Department of Restorative Dentistry and Periodontology, Faculty of Dentistry, Chiang Mai University create multilayering zirconium dioxide ceramic for dental use. The fabrication of zirconia material directly in Thailand can help reducing treatment cost and expanding treatment access for Thai patients.

One of the important factors that increase bond strength and durability between zirconia and resin cement is cleanliness of zirconia surface.[4] During try-in process, zirconia surface is contaminated with saliva. Saliva consists of glycoprotein, which formed the acquired pellicle, and phosphate group that is absorbed on zirconia surface and inhibits chemical bond with phosphate functional monomer leading to reducing in bond strength between zirconia and resin cement.[4-6] Therefore, the cleanliness of surface is very important for achieving reliable bond durability between zirconia restoration and resin cement.[5-7]

This study aimed to investigate the effect of different saliva decontamination methods on μSBS between DZ and resin cement.

Materials and Methods

Saliva was collected from one healthy female donor who had refrained from eating and drinking 1.5 hours before the collection procedure, in accordance with the Human Experimentation Committee, Faculty of Dentistry, Chiang Mai University (23/2021) and with the informed consent. Forty-two zirconia plates size 10 x 10 x 4 mm from Cercon® xt (Dentsply Sirona, USA) and DZ (Chiang Mai University, Chiang Mai, Thailand) were embedded with acrylic resin in metal ring, leaving one flat surface uncovered. The specimen surfaces were polished and sandblasted with 50 μm aluminum oxide particles at distance of 10 mm perpendicular to specimen surface at 2.5 bar pressure for 15 seconds and cleaning with distilled water using ultrasonic cleaning unit for 10 minutes. The specimens were randomly divided into 6 groups due to saliva decontamination method (n=20), as shown in Fig. 1.

Group 1 (NC): no saliva contamination

Group 2 (IC): contaminated with saliva + decontaminate with Ivoclean (Ivoclar Vivadent, Liechtenstein) for 20 seconds

Group 3 (EN): contaminated with saliva + decontaminate with 70% ethanol for 2 minutes[4]

Group 4 (SH): contaminated with saliva + decontaminate with 1% sodium hypochlorite for 20 seconds[8]

Group 5 (SB): contaminated with saliva + decontaminate with sandblasting with 50 µm aluminum oxide particles

Group 6 (W): contaminated with saliva without decontamination

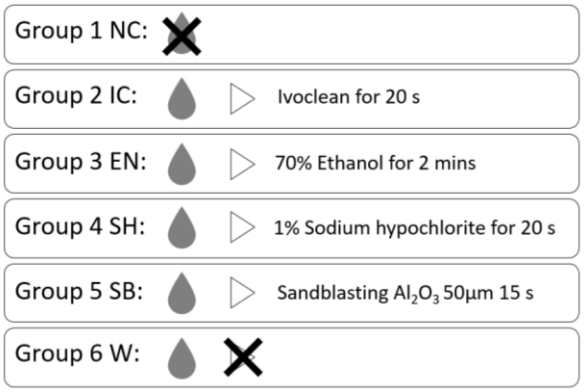

Figure 1. Experimental group of this study.

In groups with saliva contamination, saliva was applied on surface with microbrush for 10 minutes, rinsed with distilled water for 15 seconds and air-dried. In IC, EN and SH group, the decontamination solution was applied and agitated with microbrush, rinsed with distilled water for 15 seconds and air-dried. In SB group, after sandblasted, specimens were cleaned with distilled water using ultrasonic cleaning unit for 10 minutes and air-dried.

Two specimens from each group were randomly selected, first one for surface morphology examination by SEM and second one for surface elements analysis to evaluate the surface cleanability by XPS. The other five specimens were applied with primer (CLEARFIL™ CERAMIC PRIMER PLUS, Kuraray Noritake Dental Inc., Japan). Four polyethylene tubes with 0.8 mm in inner diameter and 0.5 mm in height were placed over specimen surface with clamping device, filled tubes with resin cement (PANAVIA™ V5, Kuraray Noritake Dental Inc., Japan) and light-cured with Bluephase N® LED Curing Light (Ivoclar Vivadent, Liechtenstein) in soft-start mode for 20 seconds. After cutting the tubes with blade, four resin rods were attached to the surface. All specimens were stored in distilled water at 37°C for 24 hours then subjected to µSBS test using universal testing machine (UTM) (Instron® 5566 universal testing machine, Instron Engineering Corporation, Massachusetts, USA) at a cross-head speed of 1 mm/min.

Data were collected and converted to megapascal (MPa) and analyzed using Two-way ANOVA, followed by the Dunnett's T3 multiple comparison test (p<0.05). Modes of failure were categorized into 3 groups (cohesive failure within the resin cement, adhesive failure at zirconia-resin cement interface and mixed failure) by using the SEM.

Results

The results of the XPS analysis are shown in Table 1. There were similar element distributions between NC, IC, SH and SB group. The N element concentration was found in EN and W group more than other groups. W group showed the highest C/O ratio followed by EN group then NC, IC, SH, SB group and SH group showed the lowest ratio due to high oxygen concentration on surface.

The surface morphology of different decontamination methods by using SEM did not indicate any distinctive surface characteristic between groups.

Mean µSBS and standard deviations of each group are shown in Table 2. According to Two-way ANOVA, there are no statistically significant difference (p>0.05) in bond strength between Cercon® xt and DZ in all groups, while different decontamination methods influenced the bond strength of resin cement to zirconia. Mean µSBS of NC, IC, SH and SB group were significantly higher (p<0.05) than EN and W group.

Failure mode analysis showed predominantly mixed failure in every group. Cohesive failure was found in NC, IC, SH group and SB, while adhesive failure was found in EN and W group (Table 3).

Discussion

During try-in procedure of restoration, zirconia surface was absorbed with salivary proteins with non-covalent bond and reduced the bond strength between zirconia and resin cement.[4] To solve this problem, saliva decontamination should be done prior to bonding process.

The results of this study showed that minor differences in the component of two zirconia types did not affect the bond strength to resin cement, while different decontamination methods did. This study, the effectiveness of 4 decontamination methods (IC, EN, SH, SB) were tested. The µSBS and XPS results of IC, SH and SB group showed that these methods were effective in saliva decontamination. Failure mode of this study was correlated with the bond strength. In high-bond strength groups, including NC, IC, SH and SB, showed cohesive and mixed failure, while lower bond strength groups such as EN and W showed mixed and adhesive failure.

The 70% ethanol is anti-microbial solution that usually use in dental daily practice. The intention for using ethanol as decontaminating agent is protein denaturation mechanism.[9] Due to the results of this study, ethanol seems not to be very efficient because bond strength of EN group is significantly lower (p<0.05) than others group except W group, and XPS result presenting quantity of remaining oxygen and nitrogen were higher than other decontamination methods.

Table 1. Element distribution on zirconia surface after cleansing with different saliva decontamination method (%).

Element	Atomic Concentration (%)											
	NC		IC		EN		SH		SB		W	
	C	DZ	C	DZ	C	DZ	C	DZ	C	DZ	C	DZ
C	30.61	29.16	28.33	28.52	39.03	46.77	22.39	22.46	27.46	24.87	46.58	50.29
O	45.43	44.8	47.1	44.47	37.69	32.86	49.1	50.17	48.29	47.75	30.33	26.85
Zr	7.26	8.21	10.11	10.29	5.61	4.12	9.71	9.4	8.3	9.38	3.39	3.06
N	-	-	-	1.85	6.92	4.35	0.69	-	-	-	10.44	10.21
Al	15.56	15.67	13.73	12.72	9.45	8.02	17.14	16.82	14.98	17.1	7.97	7.74

Table 2. Mean microshear bond strength and standard deviation of each group (Mean ± SD, MPa).

Group	Decontamination Method	Zirconia Type	
		Cercon® xt	Developed Zirconia
1	NC	35.13 ± 1.81^{A}	34.17 ± 1.93^{A}
2	IC	34.95 ± 3.34^{A}	36.67 ± 2.41^{A}
3	EN	26.24 ± 3.03^{B}	27.16 ± 2.70^{B}
4	SH	35.12 ± 2.02^{A}	35.54 ± 2.44^{A}
5	SB	36.10 ± 2.65^{A}	34.69 ± 2.10^{A}
6	W	28.03 ± 2.99^{B}	25.96 ± 2.76^{B}

Different superscript letters indicate statistically differences. (p<0.05)

Table 3. Failure mode of specimens (%).

Zirconia type	Storage	Decontamination Methods		Percentage Failures		
				Adhesive Failure	Mixed Failure	Cohesive Failure
Cercon® xt	Thermocycling 0 cycle	1	NC	0	60	40
		2	IC	0	70	30
		3	EN	10	90	0
		4	SH	0	70	30
		5	SB	0	90	10
		6	W	55	45	0
Developed Zirconia	Thermocycling 0 cycle	1	NC	0	70	30
		2	IC	0	70	30
		3	EN	0	100	0
		4	SH	0	70	30
		5	SB	0	85	15
		6	W	55	45	0

Airborne-particle abrasion or sandblasting is one of the mechanical surface treatment methods for zirconia which enhance the bond strength between resin cement and zirconia.[10] The result of this study showed that sandblasting can regain the bond strength after contaminated with saliva.[4,11,12] On the other hand, SEM exploration found more crack lines compare to other groups. Previous studies suggest that sandblasting on 3Y-TZP zirconia surface may cause phase transformation from tetragonal to monoclinic. Moreover, an abraded surface tends to have more crack lines which lead to decreasing in bond durability to resin cement.[13,14] Phase transformation in 5Y-TZP zirconia was not occurred due to the limit in tetragonal phase. Moreover, because of less surface hardness, the use of same particles size can roughen surface of 5Y-TZP more than 3Y-TZP, the operators should concern in size and pressure selection.[15]

Ivoclean is the popular commercial product in saliva decontamination for ceramic restoration, consists of an alkaline suspension of zirconium oxide particles. Due to the size and concentration of the particles in the medium, phosphate contaminants are much more likely to bond to them than to the surface of the ceramic restoration.[5] In this study, mean μSBS of IC group is not significantly different (p>0.05) from positive control and XPS results showed similar element distributions. Saliva consists of several chemical compounds which includes phospholipid. The structure of phospholipid includes nitrogen, carbon, oxygen and phosphate group.[16] This phosphate group is expected to incur higher chemical reaction with Ivoclean since Ivoclean contains concentrated zirconium oxide particles. This reaction may result as lower nitrogen, carbon and oxygen contents as well since these elements are attached in the same structure as phosphate group. Moreover, this type of reaction may occur with the other compounds which comprises of phosphate group.

Sodium hypochlorite has antimicrobial and tissue dissolving activities, thus it is usually used as root canal irrigant.[17] In SH group, the bond strength did not significantly different (p>0.05) from positive control group and significantly higher (p<0.05) than negative control group. The XPS result revealed that this solution could eliminate carbon and nitrogen contaminants but leave more oxygen on the surface than other groups because one substance from chemical reaction of sodium hypochlorite is reactive oxygen species that could inhibit polymerization of resin cement.[18] Even though the efficacy of sodium hypochlorite for the dissolution of organic tissue has been related to its concentration but it is high toxicity at high concentration and may cause contact irritation on soft tissue.[19,20] Also, Kim et al.[8] reported that 1% sodium hypochlorite was effective in saliva decontamination so this study decided to use 1% concentration to reduce the risk of toxicity. However, because it is commonly used in dental practice, 1% sodium hypochlorite is a good choice for using as decontaminating agent.

Conclusion

1. There was no significant difference in mean μSBS of resin cement to Cercon® xt and Developed zirconia.
2. The use of Ivoclean, sodium hypochlorite and sandblasting for decontamination were effective in regaining bond strength between zirconia and resin cement.

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OM-00007

Anesthetic Success of Adding Magnesium to Local Anesthetic in Third Molar Surgery

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ABSTRACT

Background and Objective: Despite the use of local anesthesia, patients occasionally experience pain during third molar surgery. Various approaches have been introduced to enhance local anesthetic efficacy. Adding magnesium sulfate has gained an interest as adjuvant pain treatment modality. The aim of this study was to compare the anesthetic effect of a combination of articaine and magnesium sulfate to articaine alone on the success rate of inferior alveolar nerve block in mandibular third molar surgery.

Methods: This study was a stratified, triple-blinded, randomized, placebo-controlled trial. Eighty mandibular third molars were randomly assigned into 2 groups. Magnesium group underwent conventional inferior alveolar nerve block with 4% articaine (epinephrine 1:100,000) plus magnesium sulfate (150 mg), while placebo group was administered with only 4% articaine (epinephrine 1:100,000). Both inferior alveolar nerve blocks and mandibular third molar surgeries were performed by one surgeon. The subjective signal of lip numbness, and absence of pain during the surgery were assessed. The adverse effects were also recorded.

Results: All patients reported lip numbness prior to surgery. Regarding intraoperatively absence of pain, the success rate of inferior alveolar nerve block was respectively 100% for the magnesium group, and 85% for the placebo group. There was a significant difference in success rate between two groups ($p<0.05$). None of patients reported the adverse effects.

Conclusion: The addition of 150 mg magnesium sulfate to 4% articaine (epinephrine 1:100,000) significantly increased the success of conventional inferior alveolar nerve block for mandibular third molar surgery, without any adverse effects.

Keywords: Articaine, Inferior alveolar nerve block, Local anesthesia, Magnesium, Third molar

Introduction

An adequate pain control is necessary in most dental operations including mandibular third molar surgery which is the most common surgery in a field of dentistry. In fact, the patients could feel pain during the procedure despite the use of local anesthetic.[1] Since inferior alveolar nerve block (IANB), the most commonly used for pain control in mandibular procedure, does not always achieve complete anesthesia even when administered properly.[2] Consequently, a number of approaches, including alternative IANB procedures, supplemental injection techniques, and adjuvants, have been suggested.[3] Adding an adjuvant to local anesthetic is one of the strategies that enhance the efficacy of local anesthesia. Steroid, alpha-2 adrenoreceptor antagonists, anti-inflammatory agents and other medications have been used in combination with local anesthetics including magnesium.[4]

Magnesium, an essential intracellular cation in human body function, is one of the N-methyl-D-aspartate (NMDA) receptor antagonists, which has been nominated as "the nature's physiological calcium channel blocker".[5] In physiology of pain, the activation of NMDA receptor results in central sensitization and the wind up phenomenon.[6,7] Magnesium plays a role in inhibiting this pain pathway by blocking the NMDA receptor and inhibiting calcium influx to the calcium ion channel, resulting in the prevention of central sensitization.[8-10]Because of this analgesic effects along with its cost-effectiveness and availability in both injectable and oral forms, magnesium has been used as an alternative or adjunct to alleviate intra- and postoperative pain.[8-11] Furthermore, the addition of magnesium sulfate to the local anesthetic agent has been evaluated for efficacy in peripheral nerve block and postoperative analgesic effect.[12] In dentistry, only a few studies investigated the effect of magnesium as an adjuvant for IANB and postoperative analgesia in endodontic treatment of symptomatic irreversible pulpitis.[13,14] None of the study focused on the use of magnesium added to local anesthesia for pain management in third molar surgery. Therefore, this study aimed to compare the effect of a combination of articaine and magnesium sulfate to articaine alone on the success rate of inferior alveolar nerve block in mandibular third molar surgery.

Materials and Methods

Study design and participants

This was a stratified (high and low preoperative anxiety), triple-blinded, randomized (1:1 ratio), placebo-controlled, parallel-group study. This study was approved by Ethics Committee of Faculty of Dentistry, Chiang Mai University, Thailand (protocol No. 039/2021). Participants were recruited from a pool of the patients who have intended to undergo mandibular third molar surgery at Faculty of Dentistry, Chiang Mai University. Eligible participants were all adults aged over 18 years old, who have a healthy status (ASA I

and II), and at least one impacted mandibular third molar without any inflammation or pathology that require both bone removal and tooth section. Exclusion criteria were pregnancy or lactation, allergies to medications used in this study, systemic disease contraindicated to magnesium, ongoing orofacial pain, and currently intaking of any medication or supplement.

Intervention and procedure

A web-based random sequence generator (https://www.sealedenvelope.com) was utilized to assign eligible patients (1:1 allocation ratio) to receive 150 mg of magnesium sulfate or placebo. Random permuted blocks of 4 and 6 were used with stratification according to level of preoperative anxiety (Spielberger State Anxiety score < 40 or ≥ 40). Allocation concealment was done with sealed opaque envelopes which was unsealed prior to IANB administration. The allocation was blinded to surgeons and participants until completion of the study. For magnesium arm, the participants received IANB with a mixture of 1.5 ml of 4% articaine with epinephrine 1:100,000 (Septanest SP, SEPTODONT, France) and 0.3 ml of 50% magnesium sulfate, equivalent to 150 mg of magnesium sulfate (Magnesium sulfate injection 50%, Atlantic Laboratories Corp., Ltd., Thailand) which based on previous study.[14] For placebo arm, the participants received IANB with a mixture of 1.5 ml of 4% articaine with epinephrine 1:100,000 and 0.3 ml of normal saline solution. A volume of 0.3 ml 50% magnesium sulfate or normal saline solution was drawn from an ampule using a 20-gauge needle and placed in a 3-ml syringe, depending on the randomized group. Subsequently, 1.5 ml of 4% articaine with epinephrine 1:100,000 was drawn and added into the magnesium sulfate of the aforementioned syringe. The syringe containing a mixture was inverted to mix the solution, and no precipitation was created.

Patient's preoperative anxiety was measured while patients were waiting in the reception area using Thai version of Spielberger's State-Trait Anxiety Inventory (STAI).[15] This questionnaire is divided into two parts: state anxiety (STAI-S) and trait anxiety (STAI-T), only STAI-S was applied in the study referring to a temporary emotional state related to a specific situation. The total scale has values ranging from 20 to 80, with higher scores representing more severe anxiety.[16] The STAI has no established categories, but a cutoff score of 40 has been used to identify patients with high/very high anxiety.[17] The same single oral surgeon delivered IANB and standard surgical procedure to participants. At the beginning, the IANB was administered with a blinded mixture of local anesthetic agent (1.8 ml) using 27-gauge needle, then buccal infiltration was administered with 0.6 ml of 4% articaine with epinephrine 1:100,000. Patient-reported profound lip numbness within 15 minutes of IANB administration would be considered as the criteria for IANB success.[18] After profound lip numbness, the mandibular third molar would be operated with standard surgical technique. During the surgical procedure, patients were instructed to notify the surgeon of any pain episodes. Accordingly, the success of IANB was defined as profound lip numbness within 15 minutes of administration and the tooth without pain at any stage of the procedure. The operative time (defined as the time from incision to completely wound closure) and any adverse events (nausea, vomiting, flushing of the skin, thirst, drowsiness, confusion, chest discomfort, muscle weakness) of excessive magnesium within one week after surgery were recorded. Patient with mandibular third molar on both sides, there was a minimum 4-week interval between each operation.

Statistical analysis

Based on previous study,[13] sample size calculation suggested a total of 80 mandibular third molars would detect a difference of 29% (82% VS 53%) in the success rate using a two-tailed test with a significance level of 5%, a power of 80%. Numerical data were recorded as the mean and standard deviation. Nominal data were presented with frequency and percentage. In order to test the distribution of the data Kolmogorov-Smirnov test was analyzed.

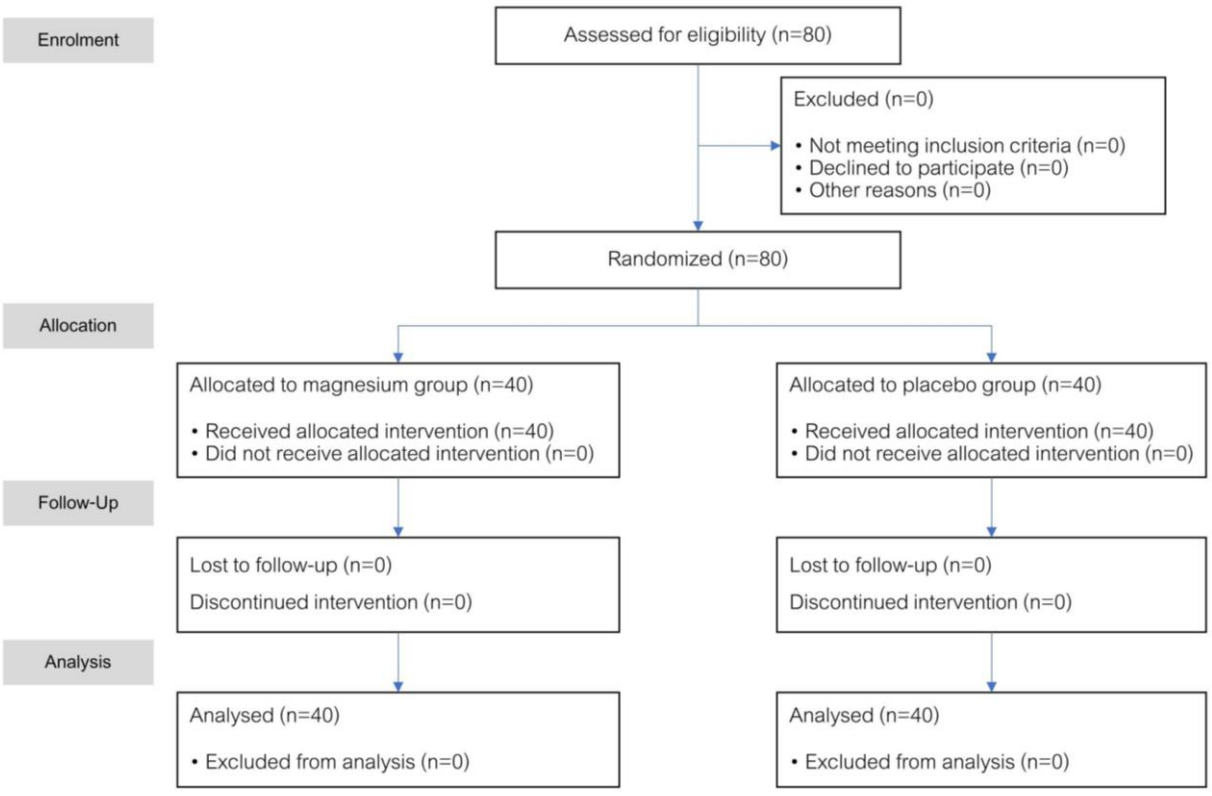

Figure 1. CONSORT flowchart.

Comparison of success rate between the magnesium sulfate and placebo group was calculated using chi square test. Comparison of mean between two groups was calculated using unpaired t-test. All data were statistically analyzed at a 95 % confidence level or *p*-value<0.05 with Stata/BE version 17 (StataCorp LLC, Texas, USA). Radiographic interpretation of mandibular third molar was calibrated with oral and maxillofacial radiologist, until Cohen's Kappa was, at least, 0.8.

Results

Eighty patients (80 mandibular third molars) were included in the study and agreed to participate. There were no significant differences in sex (p=0.49), age (p=0.41), preoperative anxiety (p=0.82), angulation of tooth (p=0.12), impaction depth (p=0.62), ramus relation (p=0.24), and operative time (p=0.13) between two groups. The study flow of all participants through the trial was presented in Fig. 1. The baseline characteristics of the patients was presented in Table 1.

Profound lip numbness was observed in all patients. However, the success rate of inferior alveolar nerve block was respectively 100% in magnesium group, and 85% in placebo group, considering patient-reported intraoperative pain. Accordingly, there was a significant difference in success rate of IANB between two groups. (p=0.01). None of patients developed magnesium-related adverse events within one week after surgery. The proportion of patients with success of inferior alveolar nerve block among groups was presented in Table 2.

Discussion

The IANB is the most frequently used and possibly the most important injection technique in mandibular third molar surgery. Unfortunately, it also tends to be the most frustrating, with the highest chance of clinical failures even when correctly administered. The success rate of IANB injections ranged from 23 to 93% from previous literatures.[19] Regarding local anesthetic agent, IANB with articaine showed anesthetic efficacy superior, or at least comparable to, other amide local anesthetics.[20] Recently, many modifications of the conventional nerve block have been described in the literature, all of which aim to achieve a high success rate and reduce the risk complications.[21] Modification of local anesthetic agent is one of the alternative strategies to optimize clinical success of peripheral nerve block. Our study showed that addition of 150 mg magnesium sulfate to 4% articaine with epinephrine 1:100,000 enhanced the success of IANB in patient undergoing mandibular third molar surgery.

Generally, magnesium sulfate has been used to treat hypomagnesemia, pre-eclampsia, and eclampsia in order to prevent seizures and is also used in the treatment of arrhythmia.[11] Magnesium has increased interest as adjunctive in the fields of

Table 1. Baseline characteristics of included patients among groups

Demographic Data	Magnesium (n=40)	Placebo (n=40)	*p*-value
Sex, n (%)			
Male	14 (35)	17 (42.50)	0.49
Female	26 (65)	23 (57.50)	
Age (years), mean ± SD	20.93 ± 2.56	21.43 ± 2.87	0.41
Preoperative anxiety, n (%)			
Low (STAI-S score < 40)	23 (57.50)	22 (55)	0.82
High (STAI-S score ≥ 40)	17 (42.50)	18 (45)	
Angulation of tooth, n (%)			
Mesio-angulation	24 (60)	17 (42.50)	0.12
Horizontal angulation	16 (40)	23 (57.50)	
Impaction depth, n (%)			
Position A	28 (70)	30 (75)	0.62
Position B	12 (30)	10 (25)	
Position C	0 (0)	0 (0)	
Ramus relation, n (%)			
Class I	0 (0)	0 (0)	0.24
Class II	31 (77.50)	35 (87.50)	
Class III	9 (22.50)	5 (12.50)	
Operative time (minutes), mean ± SD	13.85 ± 4.23	12.33 ± 4.77	0.13

*Statistically significant difference, level of significance value set at p<0.05.

Table 2. Proportion of patients with success of inferior alveolar nerve block among groups

Endpoints	Magnesium (n=40)	Placebo (n=40)	*p*-value
Lip numbness prior to surgery, n (%)			
Absence	0 (0)	0 (0)	> 0.99
Presence	40 (100)	40 (100)	
Pain during surgery, n (%)			
Presence	0 (0)	6 (15)	0.01*
Absence	40 (100)	34 (85)	
Success of IANB, n (%)	40 (100)	34 (85)	0.01*
Adverse events, n (%)	0 (0)	0 (0)	

*Statistically significant difference, level of significance value set at $p<0.05$.

anesthesia and analgesia due to a role of N-methyl-D-aspartate (NMDA) receptor antagonists. Uncertainty exists regarding the mechanism through which magnesium produces analgesia, the primary hypothesis for the analgesic properties of magnesium on peripheral nerves is the surface charge theory. A high concentration of the external magnesium concentration bathing a nerve bundle was attracted to the negative charges on the surface of the outer membrane, which had an impact on sodium ion channel gating and could lead to hyperpolarization and latter nerve conduction blockage.[22] Another possible mechanism is that voltage-dependent ion channel closes following blinding of magnesium to its specific site, inhibiting influx of calcium resulting in prevention of central sensitization from peripheral nociceptive stimulation.[9]

Numerous studies have reported the pain attenuation effect of magnesium in various surgeries such as orthopedic, cardiovascular, gastrointestinal and urogenital surgery.[23] Furthermore, the addition of magnesium sulfate to the local anesthetic agent has been evaluated for efficacy in peripheral nerve block and postoperative analgesic effect in such as axillary plexus, thoracic paravertebral, femoral, interscalene and brachial plexus block.[12] Only few studies investigated the anesthetic efficacy of adding magnesium to local anesthetic in dentistry. Our result is consistent with that reported by Mousavi and colleagues, who studied the effect of magnesium sulfate as an adjuvant to lidocaine on inferior alveolar nerve blocks in patients with symptomatic irreversible pulpitis undergoing root canal treatment.[13] They revealed that magnesium group had higher success rate of IANB at 82% and 53% for the control group. Charanya and colleagues also showed that 150 mg of magnesium sulfate when added to lignocaine provided faster and longer duration of anesthesia, maximum anesthetic efficacy, and better postoperative analgesia.[14,17] Consistently, Shetty and colleagues reported a statistically significant increase in IANB success compared to placebo in symptomatic irreversible pulpitis teeth. Their patients received two injections, the first injection was magnesium sulfate one hour prior to administration of conventional IANB as second injection.[24] Based on recent meta-analysis, adjuvant magnesium sulfate could enhance the anesthetic effects of local anesthetics and improved postoperative analgesia following the perineural blockade in most surgeries.[12] The addition of magnesium sulfate was safe in our study, which were also consistent with previous study that magnesium sulfate added to lidocaine provided an effective IANB without any adverse effects.[13]

Previous studies in dentistry mostly focused on endodontic treatment of symptomatic irreversible pulpitis, which pulpal inflammation causes local anesthetic more challenging as pulp tissue becomes more susceptible to pain.[25] Meanwhile all mandibular third molars were asymptomatic in our study, this could be the reason why our study had a higher success rate of IANB, comparing with those studies. Articaine was chosen in this study because this local anesthetic has been routinely used in our hospital and the use of magnesium sulfate as an adjuvant to articaine has not been previously reported, especially in mandibular third molar surgery. The mixture of magnesium sulfate and articaine was both physically compatible and chemically stable, similar to mixture of lidocaine in previous study.[13] The concentration of magnesium sulfate in present study was based on previous reports, which administered 150 mg magnesium sulfate or less as an adjuvant to induce nerve blocks without adverse effects and the greater success of anaesthesia,[14] and was considerably lower than the side effect-initiated dose.[26] Undesirable side effects after administration of magnesium sulfate are flushing of skin and warmth sensation. Adverse effect demonstrates as hypermagnesemia symptoms depend on serum magnesium level from mild to severe conditions including weakness, nausea, vomiting, dizziness, confusion, slurred speech, blurred vision, muscle weakness, hypotension, respiratory depression and cardiac arrhythmia. Hypermagnesemia is rare and seen most often in patients with renal impairment. Treatment of severe hypermagnesemia are airway management, cardiac monitoring and administration of intravenous calcium gluconate.[27] Nevertheless, none of patients developed magnesium-related adverse events in this study.

The removal of third molars is one of the most painful and feared dental procedures, so it is impossible to completely exclude the impact of stress on pain measurement. As a result, it is probable that stress may contribute to the complaints of pain during the surgery, which was not able to control in our study. Instead, this study evaluated individual preoperative anxiety level with STAI and found comparable STAI score between two groups, which the effect of patient's stress evenly contributed. The efficacy of local anesthesia and pain perception may be affected by both sex and age. However, there is no consensus on this influence[28] and these factors did not affect the success of IANB in this study. Surgical difficulty influences the induction of pain during the operation, that higher degree of surgical trauma from bone removal and tooth division results in more stimulation of nociceptor at peripheral nerve fiber.[29] All mandibular

third molars in this study required both osteotomy and tooth division with identical radiographic appearance. The comparable operative times between groups were observed, thus, there was similar degree of surgical trauma among groups. The experience of the oral surgeon may potentially favorably contribute to the anesthetic success of IANB established in the present study. Furthermore, the operative time required for surgical procedures was also enough for observing anesthetic success, less than 20 minutes, because pulpal anesthesia is around 1 hour for articaine.[30]

This study was designed as randomized control clinical trial to compare the anesthetic effect in both groups on the success rate of IANB in mandibular third molar surgery. The bias due to the confounding factors was minimized by the stratification of preoperative anxiety level, the randomization of treatment allocation and same surgeon performed IANB and surgery in both groups. The present study also blinded the patients, surgeon, and outcome assessor which fundamentally reduced the effect of their bias. Although, there were some limitations in our study. First, in order to combine magnesium sulfate to articaine the plastic syringe and medical needle were used instead of dental anesthesia syringe and needle. Providing IANB with this method is clinically feasible but required skill for mixing solution. Second, only one concentration of magnesium sulfate was investigated. Further study is necessary to determine alternative lesser dose of magnesium sulfate to achieve anesthetic success in third molar surgery. Third, success of IANB can be measured by both subjective and objective measurements. Nevertheless, an electric pulp test in impacted third molar was impractical and only patient-reported numbness could be done.

To our best knowledge, none of the previous studies reported the use of magnesium sulfate as an adjuvant to local anesthetic in third molar surgery. According to the findings of this study, not only endodontic procedure but also intraoperative pain during mandibular third molar surgery can be managed with a magnesium sulfate and articaine combination without the need for additional anesthetic injections, which has the advantage of using less anesthetic. Adding magnesium to local anesthetic can be one of strategy that enhance anesthetic efficacy, especially inferior alveolar nerve block, with the reasons that magnesium sulfate is widely available, simple preparation and administration, cost-effectiveness and safe. Further study in symptomatic mandibular third molar will justify more clinical benefit.

Conclusion

The combination of 4% articaine with epinephrine 1:100,000 and 150 mg magnesium sulfate significantly increase the success rate of inferior alveolar nerve blocks compared to articaine alone in asymptomatic mandibular third molar surgery, without adverse effect. Additional study is required in order to apply local anesthetics containing magnesium in daily practice.

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OM-00008

The Accuracy of Gold-Reciproc Motor as Electronic Apex Locator during Different Rotation Movements of Rotary Files

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ABSTRACT

Background and Objective: To compare the effectiveness of the Endodontic motor incorporated with apex locator, when used either with continuous or reciprocating movement.

Methods: Twenty extracted maxillary premolars, with a total of 40 root canals, were inserted with K-file until the file tip appeared at foramens and captured with a digital camera. The lengths of files were measured as 'actual length'. The samples were divided into 2 groups using two different rotary systems ProTaper Gold and WaveOne Gold. First group used Gold Reciproc motor setting auto stop function with ProTaper Gold. The final length of the file was recorded as 'preparation length'. Another group used WaveOne Gold. The difference length between actual length and preparation length of both groups were calculated and statistically analyzed with independent t-test while the difference within each group was analyzed with dependent t-test.

Results: The mean difference length was 0.14 ± 0.25 mm in WaveOne Gold group and 0.12 ± 0.27 in ProTaper Gold group. There was no statistically significant difference between two groups. In WaveOne Gold group, the preparation length was statistically different from the actual length whereas there was no difference in Protaper group. 90% of all samples distributed in range of ± 0.50 mm from the actual length.

Conclusion: The motions of rotary files were not impact the accuracy of endodontic motor device incorporated with apex locator.

Keywords: Gold Reciproc motor, NiTi file, ProTaper Gold, WaveOne Gold, Working length determination

Introduction

Maintaining working length during root canal preparation was one of the mechanical objectives of root canal instrumentation.[1] In case of losing control of working length, it could lead to instrumentation beyond or under predefined working length. Many studies showed that overinstrumentation or underinstrumentation could negatively affect the treatment outcomes.[2,3] Instrumentation beyond the apical foramen could lead to debris extrusion and injured the periapical tissue.[4] For this reason, the working length should be well controlled throughout the treatment procedure in order to prevent instruments going beyond the apical foramen.

The use of electronic apex locator (EAL) was one of the techniques to check the extent of hand file in root canal. Pagavino et al.[5] reported that the RootZX had accuracy rate of 85.5% and 100% in detecting within ± 0.5 mm. and ± 1 mm. from apical foramen respectively. This agreed with Welk et al.[6] which showed that RootZX was able to locate apical constriction in 90.7 % of all cases. Using this technique, working length was checked repeatedly from time to time to prevent the file from going far beyond the apex. During instrumentation of the root canal, the working length should be checked periodically to prevent the file going beyond the apical foramen. In order to solve this problem, endodontic motors integrated with apex locator was introduced. This equipment continuously checks the extent file while working inside the root canal and automatically stop rotating when working length is reached.

Triauto ZX (J morita, Tokyo, Japan) was an early generation of motor integrated with EAL. It showed promising results in determining the working length during instrumentation.[7,8] Recently, the new Gold Reciproc motor (VDW, Munich, Germany) has been launched and the studies on the accuracy of the equipment were done. Several studies found that accuracy of working length detection during root canal preparation was high and comparable to other apex locators.[9,10]

Currently, the movement of NiTi file rotary system could be classified into two motions which are continuous and reciprocation motion. The differences between these motions were cutting efficiency that could affect the volume of dentin removed.[11] This could result in different remaining dentin thickness around the apex during instrumentation. Therefore, the aim of this study was to compare accuracy of Gold Reciproc motor in determining the working length during the root canal preparation, between continuous rotation and reciprocation motion of NiTi rotary file systems. The aim of this study was to compare the effectiveness of the Endo motor, incorporated with electronic apex locator, when used either with continuous or reciprocating movement.

Materials and Methods

Twenty caries-free and unrestored premolar teeth with completely formed root were extracted by the reason of periodontal disease and orthodontic indication. All teeth were collected under an

ethic protocol approved by the the Institution's Ethics Committee, Srinakharinwirot University (HREC-DCU 2020-113).

All of them were stored in 0.1% thymol solution at room temperature. Root canal type and degree of curvature were ensured by radiographs of root canal separation and root curvature less than 7 degree. Crown of teeth were cut 2 mm above cemento enamel junction (CEJ) using carborundum disc. Then subjected to following measuring procedure;

Actual length measurement

K-file No.15 was inserted in each root canal. If the file failed to reach the apical portion or if the apical size was larger than K-file No.15, the samples would be excluded from the study. The "actual length" (AL) was determined when the tip of the file was visible at the apical foramen under a dental operating microscope (OPMI pico, ZEISS, Germany) at 12.5x magnification (Fig. 1). At this position, a rubber stop was adjusted to this length and held in place by flowable resin composite (Filtek Z350 flowable, 3M ESPE, USA).

Figure 1. The apical part of root canal. The arrow represented apical foramen.

Next, the file was taken out and photographed. The same position had to be maintained throughout the experiment. The distance between from the tip of the file and the bottom edge of rubber stop was measured by ImageJ software (0.01 mm precision) as AL. The measurement was done twice, the average was recorded. This study used AL as a control.

Working Model for the Accuracy Testing

Alginate powder (Kromapan, Lascod, Italy) was mixed with water according to the manufacturer's instructions. The mixture was poured into a 3x3x3 cm acrylic block with a metal clip connected to the center of block. Each root was submerged in the center of the block.

Root Canal Preparation

All 40 root canals were randomly divided into two groups according to the type of rotary file systems used ProTaper Gold (Dentsply Maillefer, Switzerland) and WaveOne Gold (Dentsply Maillefer, Switzerland) with 20 root canals each.

ProTaper Gold in F1 size (0.20/.07) was used with Gold Reciproc motor in a continuous rotary motion. The auto-stop function was activated. The machine would automatically stop when the apical constriction was detected or when the file tip reached the point specified by the motor reading. During the preparation, the canals were irrigated with 2ml of 2.5% sodium hypochlorite solution (NaOCl) and lubricated by RC-prep (Premier, USA) (Fig. 2).

When the machine stopped upon reaching the apex, the rubber stop was adjusted and fixed at the proper reference point using a flowable composite. The file was removed from the root canal and photographed at the same position where the actual length was measured. The preparation length (PL) was determined from the photos by the ImageJ software (ImageJ vers1.53e, NIH, USA). The measurement was repeated twice, and the average value was recorded. In the same manners, WaveOne Gold in Primary size (0.20/.07) was used with Gold Reciproc motor in a reciprocating motion.

The different length (ΔL) was calculated by subtracti3ng the PL by the AL of each sample. A positive value indicated that the file was beyond the apical foramen (overinstrumentation) while a negative value indicated that the file was short of the apical foramen (underinstrumentation).

Statistical analysis

The Intraclass correlation coefficient (ICC) was used to assess the accuracy of the two measurements from ImageJ software. The distribution of the data was tested by the Shapiro Wilk test. The difference between means of ΔL of ProTaper Gold and WaveOne Gold was compared using the independent t-test while the difference between AL and PL within the same group was analyzed using the dependent t-test. (Statistics 21; IBM, SPSS Inc, Chicago, IL, USA). The statistical significance was set at $p < 0.05$.

Results

Intra-observer reliability was considered excellent with an ICC of 0.928. The Shapiro-Wilk test showed that both ProTaper Gold and WaveOne Gold groups had normally distributed data.

The mean and standard deviation of length difference (ΔL) for each group were shown in Table 1. The test results revealed that ΔL between the two groups was not significantly different (p>0.05).

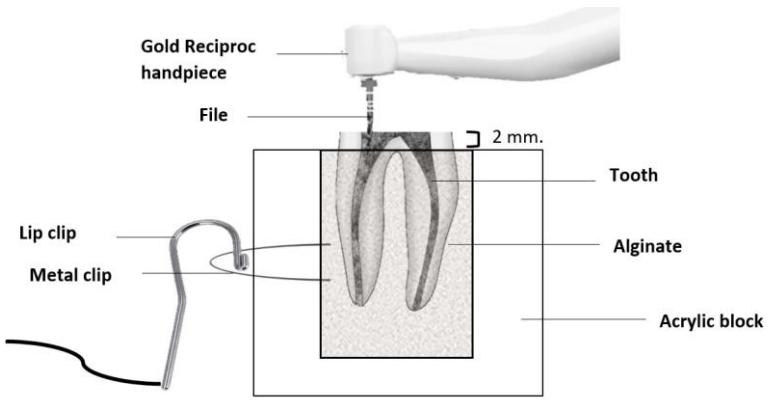

Figure 2. Procedure of preparation length measurement.

Table 1. The mean and standard deviation of length difference (ΔL) by group.

Files	Mean and SD (mm)	*p*-value
WaveOne Gold	0.14 ± 0.25	0.69
ProTaper Gold	0.12 ± 0.27	

The dependent t-test showed that no significant difference between AL and PL was found in ProTaper Gold group (p>0.05) While a significant difference was found in WaveOne Gold group. (Table 2)

Table 2. The mean and p-value of actual length and preparation length within the group.

Files	Mean Actual Length (mm)	Mean Preparation Length (mm)	*p*-value
WaveOne Gold	13.33	13.47	0.022
ProTaper Gold	13.24	13.37	0.058

When considering the location of both NiTi files, it was found that 30% of the samples in each group had the file tip short of the apical foramen whereas 70% of the samples in each group has the file tip extended beyond the apical foramen. The mean and standard deviation by file type and its location were shown in Table 3.

In addition, the experiment showed that in both groups, 90% of samples had length difference between PL and AL within ± 0.5 mm while 100% of samples had length difference within ± 1 mm from the actual length.

Table 3. The mean and standard deviation of length difference (ΔL) by the location of the file.

Files	Location	Mean and SD (mm)
WaveOne Gold	Short of apical foramen	-0.14 ± 0.17
	Beyond apical foramen	0.26 ± 0.17
ProTaper Gold	Short of apical foramen	-0.22 ± 0.17
	Beyond apical foramen	0.27 ± 0.14

Discussion

The correct determination of working length was a critical factor for successful root canal treatment as an error could lead to incomplete cleaning or injury to the periapical tissue.[12] As new endodontic motors with integrated EALs had been continuously introduced to the market, several previous studies had reported the accuracy of those devices in determining working length.[7,10,13,14] However, there were no studies reporting the effect of NiTi rotary file motion systems on the accuracy of Gold Reciproc motor.

This study applied the laboratory model as described by Higa et al.[15] using electric circuit simulation with alginate as a conductive media. Baldi et al.[16] stated that alginate was an effective embedding medium to simulate the periodontal ligament due to its good electroconductive property, colloidal consistency, firmness, and relatively low cost.

In some studies[10,17] the measurement was done by sectioning the root and then determining the distance between the file tip and the apical foramen. However, this method was technically sensitive and might cause damage to the samples. In addition, the working length was assessed by Micro-CT X-ray[18] which required small voxel size and high-resolution software. The metal-induced artefact from the endodontic file could also lead to measurement errors.[19] Therefore, like previous studies,[9,15,16] this research chose to assess the accuracy of Gold Reciproc motor by inserting the file into the root canal, measuring the preparation length from the taken photographs in ImageJ software and comparing it to the actual length because less technical sensitive, reproduceable, high accuracy in length measurement.

The outcome of this study revealed that there was no significant difference in ΔL in ProTaper Gold group. This is in accordance with the study by Christofzik et al.[10] which showed the use of Gold Reciproc with a continuous rotating file was as accurate as the use of hand file with Raypex6. Furthermore, the test by Altenburger[20] was performed using TriAutoZX with universal ProTaper file. As a result, the mean distance between the file tip and the apical foramen was 0.63 ± 0.21 mm, which was closer to actual length than previous studies. Moreover, there was no statistically significant difference from the actual length when measured to the apical foramen. Consequently, the use of Gold Reciproc with a continuous rotating file was a high accuracy.

In WaveOne Gold group, the result showed a significant difference in ΔL (p=0.22), with a mean difference of 0.14 mm The study by Wigler et al.[9] reported a high accuracy in measurements from using of the Gold Reciproc with reciprocating file. However, its preparation length was shorter than its actual length while 70% of the samples in this study extended beyond the apical foramen. Nevertheless, there was no statistical difference in terms of accuracy between the two motion systems.

The success of root canal treatment depended on cleaning and filling the root canal and could be achieved from the correct determination of working length. According to previous studies,[21,22] the ± 0.5 mm distance from the apical foramen was considered as the clinically acceptable range. On the other hand, some authors preferred the ± 1 mm distance since the constriction was not always the endpoint for of the root canal.[23] This study showed that 90% of samples in both tested groups was located within the distance of ± 0.5 mm and 100% of samples was within ± 1 mm. This was consistent with the previous studies[9-10] which suggested that using the Gold Reciproc was highly reliable for the working length determination during the root canal shaping. The results of this study therefore supported the accuracy of the Gold Reciproc with both motion systems.

Singh et al.[11] found that Protaper Gold removed more root dentine volume than WaveOne Gold. According to Al-bulushi et al.,[24] the reduction of root dentine thickness after root canal preparation related decreasing root canal impedance. Decreasing of root canal impedance might compromise the accuracy of EALs in length determination. EALs detected the distance from apical foramen by the change of impedance. When the difference was small, it resulted in interpretation to be more difficult. Considering from different amount of root dentine removed by both rotary file systems, this study showed the accuracy of Gold Reciproc when using with either Protaper Gold and WaveOne Gold were comparable in length determination. it might be expected that Gold reciproc which was the fourth generation of EALs using the ratio method, might partly overcome this problem[25].

According to this study, the Gold Reciproc showed the measurement accuracy with auto stop function upon reaching the apex. It helped maintain the working length and prevent traumatic injury to the apical tissue. The extrusion of filling beyond the root canal resulted in a decrease in the survival rate of root-treated

teeth.[26] Also, it might be difficult for teeth with significant dentin loss and unclear reference point to maintain the correct working length and possible to extend beyond the root tip. Additionally, the working length of curved canals might change after the root canal shaping by NiTi rotary files and eventually lead to extrusion.[27] Therefore, the integrated devices that could create apical stop upon reaching the apex would help maintain working length, reduce errors in measurement and increase the dentists' confidence.

Conclusion

In conclusion, there was no significant difference between continuous rotation and reciprocation in terms of accuracy of root canal length measurement by Gold Reciproc motor with integrated EALs. Both files motion systems provided highly accurate measurement.

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OM-00009

Satisfaction Level of Healthy Subjects on Mucoadhesive Film Containing *Acmella oleracea* Extract

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ABSTRACT

Background and Objective: Mucoadhesive films have been introduced as a drug delivery system for topical treatment on mucosa including in the oral cavity. Due to the anesthetic effect of *A.oleracea*, we expected that its extract can be used to develop a safe mucoadhesive film for intraoral application, to relieve pain associated with recurrent aphthous ulcer (RAU). Before being study in people with RAU, the film required the clinical study in healthy people for effectiveness, safety and satisfaction. Therefore, the present study aimed to evaluate the satisfaction level of healthy participant on the usability of film containing *A.oleracea* extract.

Methods: A total of 36 healthy participants were recruited in this clinical study. Personal information and baseline data were collected using a questionnaire. Satisfaction levels toward the product's usability including convenience, appropriate duration of adhesion and suitability of taste, were collected using numeric rating scale (NRS) in self-reported questionnaire (online google form) at 24 hours after application.

Results: The average level of participants' satisfaction was as high as 8.83 on "convenient using". The average score on satisfaction level toward the "appropriate duration of adhesion" was 7.16, whereas the average score on the "suitability of taste" was rather low at 4 of NRS.

Conclusion: After 24 hours of application, healthy participants evaluated that mucoadhesive film containing *A.oleracea* extract was at acceptable satisfaction-level on the usability, although the taste might need to be improved.

Keywords: *Acmella oleracea,* Mucoadhesive film, Oral mucosa, Satisfaction

Introduction

Recurrent aphthous ulcer (RAU) is one of the mucosal lesions, commonly found among adolescences and adults.[1] Often, the ulcer becomes painful and compromised oral-health related quality of life of the patient. Reducing pain that associated with this ulcer, has been one of the crucial aims for management of these lesions. Recent studies reported that local managements of RAU are available in various preparations, such as antiseptic mouthwashes or ointments containing anti-inflammatory or anesthetic agents.[2,3] However, their short retaining time at the site of action required patients to re-apply the medication multiple times throughout a day.[4]

Recently, mucoadhesive films have been introduced as a drug delivery system for the topical treatment on mucosa. It is capable to adhere on the specific site of oral mucosa. This allows slow releasing of the drug over a desired period time and hence, provide sufficiency of drug bioavailability.[5]

Acmella oleracea, belongs to Asteraceae family, is a native plant found in the north of Brazil, South America and Asia,[6,7] including the Northeastern Thailand which is known locally as Pak Krad Hau Weaan.[8] Many pharmacological properties of *Acmella oleracea*, have been reported, including local analgesic, local anesthetic and anti-inflammation when applied directly onto the oral mucosa.[6,9,10] *Acmella oleracea* has been classified as a herbal safety (GRAS #3783) by the Flavor and Extract Manufacturers Association (FEMA)[11] and the European Food Safety Authority (EFSA),[12] suggesting its low toxicity and a widespread usage.[13]

Based on the anesthetic effect of *Acmella oleracea*, we hypothesized that its extract can be used to develop a safe mucoadhesive film for intraoral application, to relieve the pain associated with RAU. However, as there was no recent clinical study using this preparation on healthy people, the present study aimed to evaluate the satisfaction levels of healthy adults in using the film containing *Acmella oleracea* extract on the normal oral mucosa by focusing on the usability, including convenience, appropriate duration of adhesion and acceptability of the taste before being further clinical study on people with RAU.

Materials and Methods

Study design and participants

This clinical study was conducted at the Dental Hospital, Faculty of Dentistry, Khon Kaen University and was approved by Center for Ethics in Human Research, Khon Kaen University on October 26, 2021 (Ethical number: HE641398).

Subjects were 36 healthy participants. Prior to participating in this study, the medical history was obtained. Inclusion criteria were (1) aged between 18-60 years; (2) able to sign the informed consent and answer questionnaires in online google form; (3) no inflammation or pathology on oral mucosa. Exclusion criteria: (1) known history of allergy to any components of mucoadhesive film

and/or a history of other contact allergies; (2) vulnerable participants including pregnancy or lactating women and disabilities.

After signing the informed consent, participants were instructed to apply a mucoadhesive film on oral mucosa of the lower lip and were informed to avoid drinking and eating for 30 minutes after application.

Implementation of mucoadhesive films

Mucoadhesive films containing *Acmella oleracea* extract were prepared at the size of 10 mm. in diameter, under standard procedure of the film preparation, according to pharmacological technique,[14] the ISO 17516:2014 and Thai Herbal Pharmacopoeia (THP) 2019 contamination test criteria. All films used in this study were from the same lot of preparation. Each film was sealed individually in sterile package to prevent moisture and other contamination and kept in a vacuum cabinet at room temperature. The hazardous heavy metal contamination test was performed and certified safety by Central Laboratory (Thailand) Co., Ltd. Khon Kaen Branch.

Outcomes measurements

Prior to film application, personal information on age and gender, experience of RAU and history of using any medication for the ulcer management was collected using interview questionnaire. Expected properties of the product for management of RAU in their opinion were obtained using self-reported questionnaire and opened end questions. Each participant could give more than one expected properties.

At 24 hours after application, the satisfaction levels toward the product's usability; 1. convenience, 2. appropriate duration of adhesion and 3. suitability of taste, were collected using numeric rating scale (NRS) in self-reported questionnaire (online google form).

Statistical methods

All data were analyzed using SPSS Statistics for Windows (Version 21; IBM Corp., USA). Descriptive variables were reported as percentages. Continuous variables were analyzed as means and standard deviations.

Results

A total of 36 participants were included in this study. Demographic and baseline characteristics are presented in Table 1. The majority of participants were female with the mean age of 31.48 years old. Most of the participants who had experienced RAU, also reported using topical medication, especially Triamcinolone acetonide oral paste. There was no participant reported using other medication, including mucoadhesive film/patch or systemic medication.

The percentages of each response from 36 participants (100%) were toward an opened question on "expected characteristics of products for RAU management" (Fig. 1). While 11.1% of the participants had "no particular comment", the most wanted characteristic was "reducing pain rapidly" (52.8%; 19 participants), followed by "easily using" at 30.6% and "promote wound healing" at 27.8%. The sequence of the answers was also analyzed and the results showed that "reducing pain rapidly" was the first response for 38.9% of the participants and "easily using" was the first response in 30.6% of the participants.

The levels of satisfaction toward the film after applying, were evaluated by using 1-10 numeric rating scale (NRS). The average level of participants' satisfaction was as high as 8.83 on "convenient using". The average score on satisfaction level toward the "appropriate duration on adhesion" was 7.16 of NRS, whereas the average score on the "suitability of taste" was rather low at 4 of NRS. In addition, most participants gave feedback on the taste of the film as "sweet" immediately after application and followed by continuing "bitterness" over the long duration.

Table 1. Demographic and baseline characteristics.

Characteristics	Percentage (n)	Mean (Min-Max)
Demographic data		
Gender		
Male	8.3% (3)	-
Female	91.7% (33)	-
Age		
Male	-	22.67 (22 - 23)
Female	-	31.48 (18 - 59)
Baseline data		
Experience of RAU base on participant (n=36)		
Ever	83.3% (30)	-
Never	16.7% (6)	-
Experience of RAU base on gender		
Male (n=3)	100% (3)	-
Female (n=33)	81.8% (27)	-
Used the medication to management of RAU base on ever experience group (n=36)		
Ever	63.3% (19)	-
Never	36.7% (11)	-

Discussion

The mucoadhesive film containing *Acmella oleracea* extract as the topical pain reliever has been successfully developed and tested for drug delivery properties *in vitro* by our interdisciplinary team at the Khon Kaen University.[14] The crucial *in vitro* findings on drug releasing rate and adhering duration to the mucosa, were promising and suggested high potential for clinical use in patients. In this initial clinical study, healthy participants were recruited and the films had been applied on normal oral mucosa, in order to focus on the outcomes of the satisfaction on applying the film.

Studies have illustrated that the lifetime prevalence rates of RAU experience was in the range of 28.1% and 36.5%,[15,16] with a higher prevalence in female. In Thailand, the prevalence of RAU reported among dental patient population since 1991, was found to be 46.8% (n=1,451) while the experience of having the ulcer were 44.5% among 11-20 years old group and decreased to be 24.5% in the 21-30 years old group.[17] RAU could be classified into three clinical types, minor type, major type and the herpetiform type. They differ in morphology, distribution and severity. Altenburg et al. reported that small aphthous ulcer could be healed spontaneously within 4–14 days.[3] In the present study, 83% of participants reported having experience of RAU, however, they could not specify the type of RAU. Interestingly, more than 60% of those who had the experienced reported using medication to manage their RAU. It could be speculated that the participants who chose to use medication, hoped to alleviate the pain associated with the ulcer. This was consistent with the result that to quickly relieve pain was the most anticipated characteristics of the product for management of RAU.

Considering the pain management of oral ulcer, the mucoadhesive film *per se* seems to be beneficial to protect the mucosal ulcer from further environmental irritation, by which the ulcer was covering by the film.[18] Additional advantages of the films were flexibility and adaptability, which provide greater comfort to apply onto the sensitive sites for a certain duration, to ensure that drug releasing is sufficient.[19,20] Whereas the noticeable disadvantages of the topical oral paste/gel applied directly onto the mucosa, are the retention of medication, which affected by the salivary flow and regular movement of oral muscle. As the concentration of medication reduced, the therapeutic effect might be decreased,[21] so patients are required multiple application per day.

In Thailand, several medications for oral ulcer in various preparations are available and affordable over the counter. The only preparation used among the participants was the topical administration in the form of oral paste or gel. None of the participant in this study reported using mucoadhesive film/patch or herbal product for management of RAU. From the user's perspective, we found that the properties required from product for the

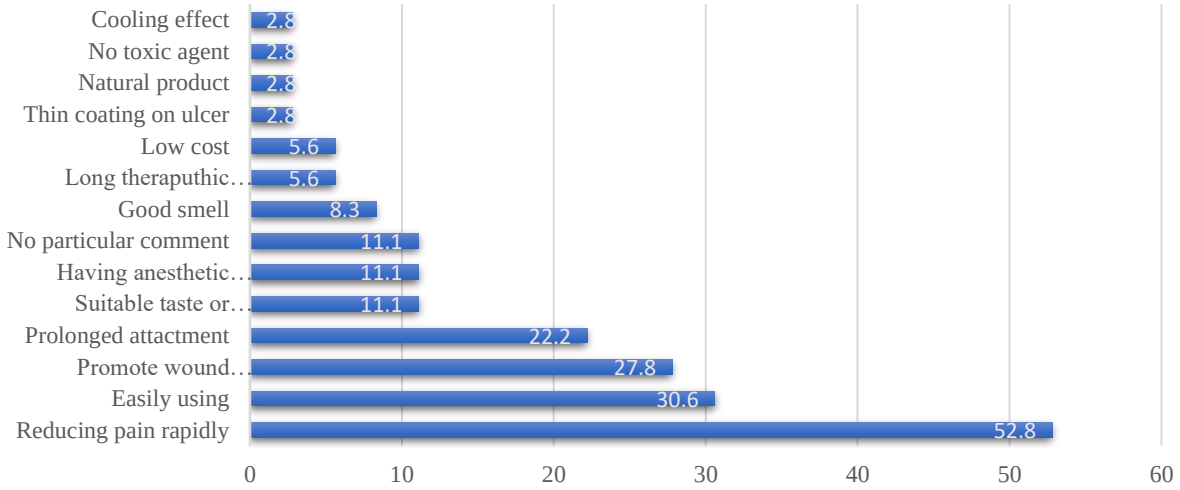

Figure 1. Diagram shows the percentage of expected characteristics of the products for RAU management responded in the participant's self-report.

management of RAU were reducing pain rapidly, easily using and low cost, following by prolonged attachment, long duration of therapeutic effect, tasteless and cooling. These suggested characteristics aligned perfectly with our direction for further improvement of the products.

Participants rated "the convenience in using" at the satisfaction level above 7 on NRS. This finding demonstrate that the tested film was easy to use for intraoral application, consistent with other previous studies.[2,22] The contact period with oral mucosa has been suggested as another crucial parameter of drug delivery system.[22] The actual duration of the film retention at the lower lip could be varied depending on the person's activities, especially during speaking or eating. The tested site was selected because this region was found frequently to have the recurrent aphthous ulcers.[23] Most participants rated more than level 5 of NRS, demonstrating that the intraoral adhesion duration was appropriate.

The NRS scores on "suitable taste" was at the lowest level. Bitterness derived from the releasing of *Acmella oleracea* extract, the active ingredient for analgesia. Data from the questionnaire also showed that many participants explained the experience of brief sweetness of the xylitol coated, occurring immediately after film application. Although the overall satisfaction on taste remained poor, there were relatively few participants who highlighted the importance of the flavor of the product. Anyhow, the taste will be one of the main characteristics to be improved as it could affected the overall clinical performance of the film.

The present study evaluated user's perspective on the usability of the new product and recruited only healthy participants with no oral ulcer. However, most participants enrolled in the study had reported previous RAU experiences and majority of them had used oral paste for pain management. Therefore, their opinions regarding the wanted characteristics of mucoadhesive film for pain management, given in the self-reported questionnaires, served as a useful information for product improvement.

Main outcome of this study was the satisfaction levels of the users, which was a subjective outcome. The interpretation of the questions and their responses mainly depend on the individual's experience. The numeric rating scale (NRS) has been successfully used in other similar subjective studies on satisfaction and has showed to be more accurate and was easier to understand for various clinical subjective measurements.[24,25] Numeric rating scale using in this study was, therefore, appropriate and informative for the objective of study.

The next appropriate study, we are currently conducting a randomized control trial to evaluate the effectiveness of local anesthetic effect and safety of this film in the large numbers of healthy participants (Clinicaltrial.gov with NCT05219747 identifier).

Conclusion

The satisfaction of healthy adults in using the film containing *Acmella oleracea* extract on the normal oral mucosa were, at least, at the acceptable level, on several aspects including measurement of convenience and appropriate duration of adhesion. While the acceptability of the taste may need to be improved in the future.

Table 2. The average scores of satisfaction levels on mucoadhesive film application, assessed by numeric rating scale (NRS).

Characteristics of Satisfaction	NRS Score		
	Mean (SD)	Median	Interquartile Range (Q1-Q3)
Convenient using	8.83 (1.64)	9	8.25 - 10.00
Appropriate duration of adhesion	7.16 (2.24)	7	5.25 - 9.00
Suitability test	4.00 (2.61)	3	2.25 – 5.75

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OM-00010

Expected Barriers in Providing Geriatric Dental Care in Thailand: Questionnaire Validation Study

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ABSTRACT

Background and Objective: This pilot study assessed the validity and reliability of a Thai version of a questionnaire about geriatric dental care's barriers developed by Hatami and colleagues. This questionnaire can be used to investigate dental students and dentists and suggest strategies to improve geriatric dentistry education in Thailand.

Methods: The 8-item questionnaire made by Hatami and colleagues was translated to Thai language. The translated version was pretested by 29 dental students and necessary adjustments were made. A pilot study was conducted with dental students and dentists at Chulalongkorn University to evaluate internal consistency of the questionnaire.

Results: The introduction sentence of the questionnaire was adjusted to shift the point of view from self-perception to objective expectation about barriers to include undergraduate students with no geriatric clinical experience in the investigation. A nineth barrier regarding lack of caregiver's awareness in caring for elderly patients' oral health was added. Description of each score in the 5-point Likert scale was changed to ease understanding, ranging from 1 = "not a barrier" to 5 = "be a big barrier". 130 persons participated in the pilot study. Cronbach's α for internal consistency was 0.813. The added barrier received the highest percentage of scores 4 and 5. Preliminary results showed differences in barrier scores between participants' subgroups.

Conclusion: The research instrument was valid and reliable. The change in point of view and inclusion of clinically unexperienced students allowed comparisons among undergraduate sub-groups. The added barrier was correlated with other items and received deep concerns from the participants. Further investigation should clarify association between barriers and participants' characteristics and find comprehensive solutions for a better geriatric dental care provision experience.

Keywords: Dental care, Geriatric, Questionnaire validation

Introduction

Thailand, home to 69 million people, is the second most "aged" country in the Association of Southeast Asian Nations, only behind Singapore. With a rapid increase in the elderly population rate of about 3.5% per year, in 2022, Thai elderly population is expected to reach 20%, making the country become a "complete-aged country". In two decades, the million-birth cohort born between 1963 and 1983 will all become older adults, raising the elderly proportion even higher to nearly one in three.[1]

A national oral health survey conducted in 2017 revealed complicated oral health conditions among the Thai elderly.[2] The major problem for elderly patients was tooth loss, especially complete tooth loss, seen in 8.7% of elders aged 60-74 years and up to 31.0% of the age group of 80-85 years. In addition, 52.6% of the Thai elderly had untreated caries, 16.5% had root caries associated with gum recession, and 36.3% had periodontitis, of which 12.2% were at a very severe level (with a probing depth of 6 mm or more). Hence, dental students and dentists in Thailand need to be prepared for the increasing need for dental care and the compromised oral health status of the elderly population.

However, there have been expected barriers in treating elderly patients among dental students and dentists, which affect their attitudes and readiness to provide treatment to the old.[3-5] A research of Chua and colleagues in a similar subject group which was medical students revealed that students' attitudes towards treating the elderly were strongly associated with their future career preference in the field of geriatric medicine, reinforcing the need to improve students' willingness in treating this frail population by helping them address and resolve any related obstacles.[6]

To evaluate barriers to provision of dental care to elderly patients, Nitschke and colleagues and Hatami and colleagues developed two questionnaires with different lengths and designs.[3,5] While Nitschke and colleagues employed an open-ended questionnaire with 36 statements which was suitable for semi-structured interviews, Hatami and colleagues, on the other hand, developed an 8-item closed-ended questionnaire.[3,5] The two studies led to varied results, one reason being the the fact that obstacles were based primarily on the environment and circumstances of each country. A study examining those challenges in the context of Thailand is fundamental in giving an insight into the current situation and recommending how undergraduate dental curriculum can be developed to help students manage the expected obstacles and promote confidence in treating geriatric patients.

In order to develop a research instrument evaluating expected barriers in providing geriatric dental care in Thai language, we translated the questionnaire developed by Hatami and colleagues[3] and conducted a study to assess the cross-cultural adaptation,

validity and reliability of the translated version. As we planned to distribute the questionnaire in an online form with no interview involved, the questionnaire of Hatami and colleagues was considered to be the optimal choice due to its reasonable length, closed-ended question design and accessibility.

Materials and Methods

The study protocol was approved by the Human Research Ethics Committee at the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2021-094).

Research Instrument

The research instrument of this study was based on an 8-item questionnaire by Hatami and colleagues investigating dental students' perceived barriers in geriatric dental care. The questionnaire was developed based on guidelines in geriatric dentistry and expected outcomes of geriatric dentistry education.[3] Its content validity, face vadility, and reliability was evaluated by its developers.[3]

We informed the developers of the perceived barrier questionnaire and asked for their permission to translate the questionnaire into Thai language via the corresponding author's email reported in the original questionnaire development study.[3]

In this study, the introduction question was adjusted from "How do you assess the importance of each item in hindering your providing dental care to geriatric patients?" to "How do you assess the importance of each item in hindering the provision of dental care of a dentist in general to geriatric patients?" to make it easier for dental students who hadn't experienced treating elderly patients to express their expected barriers when treating the elderly patients. As a result, this adjustment changed the point of view from self-perception to expectation about severity of possible barriers seen by a dentist in general in the context of Thailand.

Translation and Cultural Adaptation

The development of the Thai-translated version of this research instrument followed guidelines for the process of cross-cultural adaptation of self-reported questionnaires by Beaton and colleagues[7] Two forward translations of the instrument (FT-1 and FT-2) were made independently from English to Thai by two Thai translators, one of them being a senior dental student and the other being expert in English translation. A meeting was held for the two translators to create a synthesis of these translations (FT-12). Next, back translation of the FT-12 version was done separately by two English native speakers who were fluent in the Thai language (BT-1 and BT-2) to evoke unexpected meanings of the items and to check the validity of the questionnaire. An expert committee was held to consolidate all the versions of the questionnaire. The committee included a Thai prosthodontist who had more than 20 years of experience in the field of geriatric dentistry, a Thai assistant professor expertizing in teaching English as a second language, and all the forward and backward translators. After examining all the translations, we managed to develop the pre-final version of the questionnaire with satisfying semantic, idiomatic, experiential, and conceptual equivalence with the original questionnaire of Hatami and colleagues.[3]

The translated version was then pretested by a group of 29 undergraduate and postgraduate dental students at Chulalongkorn University, Bangkok, Thailand for linguistic validation and content validity evaluation. Participants were asked to answer the questionnaire and give opinions about how they interpreted each questionnaire item and the equivalent response. When issues with comprehension and appropriateness of wording arose, necessary adjustments were made accordingly. An open-ended question was also included, asking about other possible barriers to the provision of dental care to the elderly.

Adjustments of The Questionnaire

Following the linguistic validation and content validity evaluation, comments of the pretest participants were collected. An expert meeting was held taking into consideration participants' opinions and suggestions of specialists in geriatric dentistry. As a result, necessary adjustments were made to the questionnaire. The adjusted version of the questionnaire was resent to the pretest group for re-assessment of face validity.

Pilot Study Evaluating Reliability of The Expected Barrier Questionnaire

The adjusted questionnaire was designed in an online format and a pilot study was carried out in the Faculty of Dentistry, Chulalongkorn University by sending the questionnaire at the same time to LINE groups of undergraduate students from every year of study (1st year to 6th year), postgraduate students, and dentists currently working at the university. Dental students and dentists were invited to answer the questionnaire after agreeing with an online consent form which included the information about the purpose of the study, which is to evaluate cross-cultural adaptation, validity and reliability of the expected barrier questionnaire.

According to a recent guideline, a sample size of 7 times the number of items and at least 100 participants was considered very good for a pilot study testing the cross-cultural validity of a questionnaire.[8] With an additional 20% drop-out compensation, our pilot study's required sample size was 120.

In addition to the study instrument, the questionnaire asked about background details including gender, current academic standing (undergraduate student, post-graduate student, or dentist). Undergraduate students were asked one additional question about whether they had had experience in treating elderly patients.

The open-ended question asking about other possible barriers to the provision of dental care to the elderly was also used for this participant group.

Statistical Analysis

The demographic data of the participants were presented descriptively. The research questionnaire responses were scored using a 5-point Likert scale. Cronbach's α was calculated to estimate internal reliability of the questionnaire. The normality test was conducted, resulting in expected barrier scores among sub-groups not in a normal distribution (p<0.05). Median, first and third quartile (Q1-Q3) score values were determined for each item of the questionnaire. The significance level was fixed at 0.05 for the Mann-Whitney U test, Kruskal-Wallis H test, and Dunn's test. SPSS statistical software was used for all statistical analyses (version 22.0, IBM Incorporated, NY, USA).

Results

Translation and Cross-cultural Adaptation

The expected barrier questionnaire was translated into Thai language. All versions of the translation (FT-1, FT-2, FT-12, BT-1, ant BT-2) and the original version were equivalent.

Linguistic Validation and Content Validity Evaluation and Adjustments of The Questionnaire

We conducted an investigation asking participants' interpretation of the expected barrier questionnaire. Although we stated in the questionnaire introduction sentence that participants needed to take the point of view of a dentist in general to evaluate the severity and each barrier in the context of Thailand, from our investigation of participants' interpretation of the introduction sentence, some still misunderstood that they should answer based on their own perceived barriers. In the effort to get rid of this misleading idea, we further adjusted the introduction phrase to "In general, how big of a barrier do you think each of the following scenarios would cause to a dentist in treating elderly patients?" with an

accompanying explanation of taking into consideration the current situation in Thailand.

In addition, the word "important barrier" seemed confusing when being translated to Thai language, so we adjusted the meaning of the answer choice range from 1 to 5 as follows:

1 = "not at all" was changed to "not a barrier"

5 = "very important" was changed to "be a big barrier"

Also, items of the questionnaire were noun phrases among which some mentioned only the barrier factors, such as "follow up of elderly patients", "elderly patient's compliance", financial ability of elderly patients" while others were accompanied with words illustrating difficult scenarios, for instance, "inadequate self confidence in elderly patient management" and "inadequate knowledge of geriatric dental care", which led to confusion and difficulties in answering the questionnaire. Hence, the items were rephrased into full statements to make participants easier to interpret.

In terms of answers regarding other possible barriers in providing geriatric dental care, the most common opinion was "caregivers' lack of awareness in taking care of the elderly patients' oral health" (7 out of 29 answers), while the other comments were rather detailed explanation of the listed items, such as "hearing problems" which was related to patient management or "compromised systemic diseases" requiring adequate knowledge in geriatric dental care. As a result, one more statement (statement number 9) was added asking about caregivers' awareness in caring for older adults' oral health. The content validity of the adjusted questionnaire was assessed by academic staff and dentists who are experienced in geriatric dentistry. The adjusted questionnaire presented below was resent to the pretest group and all the participants found it to be clear and easy to understand.

"In general, how big of a barrier do you think each of the following scenarios would cause to a dentist in treating elderly patients?"

1. The dentist doesn't have adequate self-confidence in elderly patients' management
2. There are difficulties in following up on the elderly patient's symptoms
3. The elderly patient doesn't cooperate
4. The dentist doesn't have adequate knowledge of geriatric dental care
5. The elderly patient has difficulties in paying for their dental treatment
6. The elderly patient takes a long time or has difficulties in traveling to a dental clinic
7. The dentist has inadequate communication skills with elderly patients
8. The dental office lacks appropriate facilities for treating the elderly
9. The caregiver doesn't put enough importance on caring for the elderly's oral health

As aforementioned, the responses were categorized on a Likert scale (1=not a barrier, 2=be a small barrier, 3=be a barrier, 4=be a rather big barrier, 5=be a big barrier).

Pilot Study Evaluating Reliability of The Expected Barrier Questionnaire

The online questionnaire received 131 answers, of which 1 responder refused to accept the consent form, making it 130 valid. The median, first and third quartile values of participants' ages were 23 (21-25). Table 1 illustrates the demographic characteristics of dental students and dentists. Among them, 61.5% (n=80) were female and 38.5% (n=50) male. The majority of respondents were undergraduate students (79.2%, n=103), while 13.1% (n=17) were postgraduate students and 7.7% (n=10) were dentists working at the Faculty of Dentistry, Chulalongkorn University. Less than half of undergraduate students (48.5%) had treated elderly patients during their practice in the university program.

Table 1. Demographics of participants.

Characteristic	Number	Percentage
Gender		
Female	80	61.5
Male	50	38.5
Current position		
Undergraduate	103	79.2
Postgraduate	17	13.1
Dentist	10	7.7
Having treated elderly patients (Undergraduate students)		
No	53	51.5
Yes	50	48.5

For almost all the items of the questionnaire, the percentage of scores 4 and 5 was higher than that of scores 1 and 2, except for the barrier regarding financial difficulties (Fig. 1). This showed that the participants were generally aware of barriers in treating elderly patients. Lack of caregivers' awareness in taking care of older adults' oral health received the highest percentage of scores 4 and 5.

Cronbach's α is a well-known parameter which indicates the correlation between items in a research instrument.[9] Due to the small sample size of postgraduate students and dentists, Cronbach's α was calculated for all the participants as a whole group. The expected barrier questionnaire in this study achieved a Cronbach's α of 0.813. The corrected item – total correlation values of 9 items of the questionnaire ranged from 0.249 to 0.620, with the average value of 0.509. The added barrier about caregivers' lack of awareness in taking care of elderly patients received the corrected item-total correlation value of 0.552. Cronbach's α would be reduced to 0.789 if this item was removed.

There was no significant difference between male and female participants for all the barriers. Undergraduate and postgraduate students had significantly deeper concerns about the lack of treatment facilities for the old compared to working dentists (p=0.010, Kruskal-Wallis H test; p=0.020 and 0.010 respectively, pairwise comparisons using Dunn's test). Among undergraduate students particularly, those who haven't had previous experience in treating older adults put significantly more importance on various barriers compared to those who had treated elderly patients (Table 2).

Participants also gave opinions about other possible barriers, including "Dental assistants lack cooperating skills", "Complicated referring system makes it hard for older adults to use their health insurance", "The elderly patient doesn't have caregivers to take him/her to the dental clinic or to take care of his/her oral health", and "The elderly patient has a faulty belief about his/her oral health or is unaware of the need to take care of it".

Discussion

The translation and cross-cultural adaptation process of the questionnaire to Thai language followed a standardized protocol to ensure an acceptable equivalence with the English version. The translated version was pretested with a group of 29 dental students and dentists, and any reported issues with understanding the questionnaire were addressed by adjusting the questionnaire accordingly. Content validity was rechecked by experienced dentists and academic staff. According to pretest participants, the updated version was clear and easy to understand which proved adequate face validity.

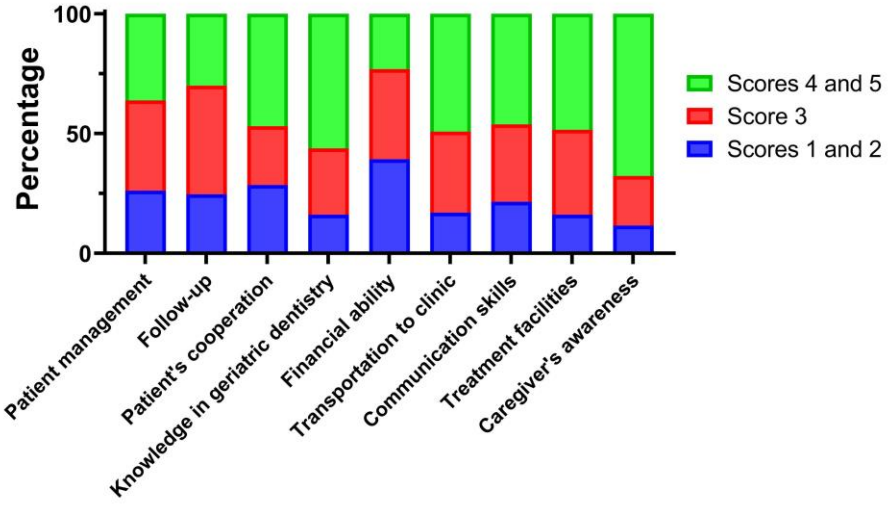

Figure 1. Percentages of answers regarding expected barriers in treating elderly patients.

Together with senior dental students and dentists, this study also included preclinic dental students who had not had any experience in treating patients. This was not the case for previous studies, where all participants are certified dentists or senior dental students with at least some exposure to providing dental treatments.[3,5] This change of inclusion criteria enabled a comparison in expected barriers in geriatric dental care between dental students who had experienced treating the elderly and those who had not had the chance, so a proper approach to dental teaching can be adopted as soon as dental students start their first years of study in the field of dentistry. Preliminary findings showed that dental students with no exposure to geriatric dental treatments reported significantly deeper concerns regarding various barriers compared to experienced classmates or seniors. This confirmed the need for further investigation about possible differences in undergraduate student groups' opinion and expectation regarding obstacles in providing dental care to older adults.

As an implication of the inclusion criteria in the study, it was needed to shift the point of view of participants from self-perception to objective expectations about barriers in geriatric dental care that were worth concerning. With this approach, it was impossible to know whether and how often these barriers happened to dental practitioners. But on the other hand, it would give an insight about which barriers, when happening, would be expected to have a great impact on the process and outcomes of the dental treatments. For instance, a dentist might see poor elderly patients very often, but he/she can always come up with an affordable treatment plan which can most of the time be paid by the patients' health insurance, so he/she would rate financial difficulties as not a significant barrier. This objective approach would be beneficial in suggesting adjustments of the geriatric dentistry curriculum focusing on what mattered that could really increase the quality of the treatment and positive experience of dental practitioners.

The present study used Cronbach's α to determine internal consistency. A Cronbach's α of at least 0.70 indicates an acceptable internal reliability of the questionnaire.[9] In this study, the Cronbach's α score of 0.813 proved that the 9 statements listed in the adjusted questionnaire were related to the same topic: barriers in geriatric dental care.

In addition to the 8 items listed in the original questionnaire, a nineth barrier was added regarding caregivers not putting enough importance on caring for the elderly's oral health as it was considered by many pretest participants as a big obstacle in geriatric dentistry practice. Questions were raised regarding the necessity to include this factor in the questionnaire for participants to rate its severity in comparison with other items. Acknowledging that lack of caregiver's awareness in caring for elderly patients' oral health can lead to other problems listed in the questionnaire such as transportation to dental clinic, patient's follow-up, or patient management, inclusion of this factor would require participants to shift their point of view from the elderly patients, dentists, or dental facilities to the caregivers. Hence, adding this barrier to the questionnaire would help ease data analysis

Table 2. Median, first and third quartile (Q1-Q3) values of expected barrier scores in providing geriatric dental care among undergraduate students according to previous experience in treating elderly patients (1 = not a barrier; 5 = be a big barrier).

Barrier	Having Treated Elderly Patients (Undergraduates)		
	No	Yes	*p*-value#
Patient management	3 (3-4)	3 (2-4)	0.005**
Follow-up	3 (3-4)	3 (2-4)	0.018*
Patient's cooperation	4 (3-5)	3 (2-4)	0.004**
Knowledge in geriatric dentistry	4 (3.5-5)	3 (3-4)	<0.001***
Financial ability	3 (2-3)	3 (2-4)	0.234
Transportation to clinic	3 (3-4)	4 (3-4)	0.625
Communication skills	4 (3-5)	3 (2-4)	0.018*
Treatment facilities	3 (3-4)	4 (3-4)	0.673
Caregiver's awareness	4 (3-5)	4 (3-4.25)	0.474

Values are presented as median (first quartile-third quartile)
Mann-Whitney U test; * significant (p<0.05); ** significant (p<0.01); *** significant (p<0.001)

and interpretation, leading to evidence-based explanation for prominent causes that hinder dental students and dentists' provision of geriatric dental treatments. Considering that the corrected item – total correlation value of this barrier was higher than the threshold value of 0.30, it demonstrated good correlation with other items of the questionnaire. In addition, as shown in the results of this study, this barrier received particularly deep concerns among participants. Further research should be conducted to explore how different barriers are related in order to form a comprehensive strategy to solve all those problems as a whole.

Differences in opinions were seen between participants' subgroups based on their current position. Undergraduate and postgraduate students were significantly more concerned about lack of appropriate facilities compared to dentists. Due to the small sample size of this pilot study, this finding should be judged with cautions, and future community survey can clarify this association together with plausible causes, so treatment facilities can be distributed adequately for those in need.

There existed limitations of this study that should be noted. As the data collection process was done online with no interview involved, the feasibility of asking for individualized opinions of each participant was challenged. Although we tried to send the questionnaire to the participants at the same time, they might have seen and answered the questionnaire at different times up to their comfort, which might be another confounding factor. Future research should be conducted with more participants from different parts of Thailand in form of a community wide survey, so the research sample would represent the population of dentists in Thailand, and a more detailed explanation of barriers in geriatric dentistry would be collected with the use of open-ended questions. Once the association of dental students' and dentists' characteristics and their expected barriers are found systematically, an individualized approach in the geriatric dentistry education can be applied to meet students' need and improve their geriatric dental care experience.

Conclusion

The Thai version of the expected barrier questionnaire proved evidence of validity and reliability and can be used in dental students and dentists for further investigation in the field of geriatric dentistry. The change in point of view of the questionnaire and the inclusion of unexperienced dental students led to new findings and the feasibility to compare among undergraduate students' subgroups. The added factor regarding caregivers' lack of awareness in taking care of older adults' oral health was correlated with other barriers and allowed participants to express their deep concerns in this matter. Further research with a bigger sample size might clarify association between dental students' and dentists' characteristics and their expected barrier scores and look for comprehensive ways to improve geriatric dental care experience, giving way to more effective geriatric dentistry education and successful dental care to older adults in Thailand.

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Translation and Cross-cultural Adaptation of the Thai Version of OHIP-TMDs

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ABSTRACT

Background and Objective: Patient-reported outcome measures (PROMs) of quality of life have become an outcome of interest in the management of temporomandibular disorders (TMD). The Oral Health Impact Profile for TMD (OHIP-TMDs) is a condition-specific quality of life (QoL) measure, specifically for TMD. This study aims to translate OHIP-TMDs and perform a cross-cultural adaptation of the Thai translation.

Methods: The protocol of cultural adaptation was adapted from Beaton et al. as follows: (1) the forward translations were performed by the author and a Thai occlusion and orofacial pain specialist and were synthesized into one translation text by another occlusion and orofacial pain specialist; (2) Back-translation was created by one native English speaker; (3) The review by a committee of experts and the original author were prepared to create a prefinal version; (4) A pre-final instrument was tested and discussed with thirty Thai TMD patients to ensure understanding.

Results: The committee of experts reviewed all the texts that were created following the translation protocols and developed a clear and easy-to-understand Thai version. A questionnaire was fully approved by the original author. The pretesting was done with twenty-three female and seven male TMD patients. The Cronbach's alpha coefficient was 0.961, demonstrating an acceptable internal consistency.

Conclusion: The Thai version of OHIP-TMDs proved to be understandable, clear, and applicable. It successfully transculturally adapted for future validation and application in Thai TMD patients.

Keywords: OHIP-TMDs, Oral health-related quality of life, Temporomandibular disorders

Introduction

TMD are orofacial pain problems that involve masticatory muscles, temporomandibular joint (TMJ), and head and neck musculoskeletal structures. The patient's symptoms are tenderness and pain in the masticatory muscles, pain in the TMJ, limited jaw movements, and clicking or crepitus sound on the TMJ.[1] Dental practitioners are challenged with patient pain control and clinical evaluation of TMD. Pain is a personal subjective experience that can negatively affect the individual and cannot be measured objectively. Patient-reported outcomes (PROs) are defined as "a patient's health condition that is reported directly from the patient, without any interpretation by a clinician or any other person" and have been researched to use for measuring the presence of pain in general in dentistry.[2] Dentists and practitioners can better understand what matters to patients across the domains of quality of life that are affected by pain using PROMs. It is controversial for physicians to choose between active or passive treatment modalities and to evaluate patient adherence to certain treatment modalities that may be influenced by psychological variables. Such domains will also impact patient compliance and treatment adherence, which will impact the overall disease prognosis.[3]

Many studies showed that TMD have a negative impact on QoL.[4,5] Therefore, measurement of QoL could be considered an important outcome in the management of TMD.[6] We can better identify which outcomes and domains are important to patients by measuring their perspectives on pain and treatment experience. PROMs, in terms of questionnaires, are based on the examination of patient experiences.[7] The well-known QoL measurement in dentistry is a full 49 items of the Oral Health Impact Profile (OHIP-49), which is a famous measurement that has been used for various conditions and diseases and has also been shown to be sensitive to change in TMD.[8] Even though several authors have advised using OHIP-49 for TMD, major drawbacks of this instrument are total number of questions which is time-consuming. Therefore, employing a shorter item or a disease-specific instrument should better be chosen. For evaluation of symptoms change or disease progression over time, using the disease-specific instrument will increase the instrument's sensitivity and discriminatory ability.[9,10]

Durham et al. in 2011 developed a condition-specific QoL measure specifically for TMDs using a combined quantitative-qualitative methodology to identify the most relevant items from OHIP-49 for TMD.[11] A variety of techniques are available to determine or interpret scores of the instrument,[12] but no method has demonstrated advantages over another.[13] The simplest technique (OHIP-ADD) creates a summary score for the entire instrument by adding the ordinal response scale response codes for each item across all domains. The results of validity, reliability, and responsiveness to change test of OHIP-TMDs are good. And because it is shorter and contains information especially related to TMDs, OHIP-TMDs is more useful and convenient than OHIP-49 for TMD patients.[14]

OHIP-TMDs showed comparable internal reliability to OHIP-49 and had less than half the number of items as OHIP-49 which help lowering the response load and potential attrition and acquiescence bias in cohorts of TMD patients. OHIP-TMDs has been translated into

other languages, such as a Chinese version; the OHIP-TMDs-C,[15] and has been used for determining the associations between TMD chronicity and oral health-related quality of life.[16] Because OHIP-TMDs had not yet been translated to the Thai language and cross-culturally adapted for use in Thailand. Therefore, this study proposes to translate and perform a cross-cultural adaptation of OHIP-TMDs. For the ultimate goal, we believe that OHIP-TMDs could be a promising PROMs instrument for Thai TMDs patients and help improving orofacial pain research and management.

Materials and Methods

This study received the approval of the Human Research Ethics Committee, Faculty of Dentistry, Chulalongkorn University, Thailand (HREC-DCU 2021-072). All participants provided written informed consent before entering the study.

OHIP-TMDs consists of seven domains: 1) functional limitation, 2) physical pain, 3) psychological discomfort, 4) physical disability, 5) psychological disability, 6) social disability, and 7) handicap. The response is recorded on a five-point ordinal scale of frequency of the situation: never (0 or best possible QoL), hardly ever (1), occasionally (2), fairly often (3), very often (4 or worst possible QoL).[11] The translation and cross-cultural adaptation of OHIP-TMDs into the Thai language were adapted from the internationally accepted process established by Beaton et al.[17] and Guillemin et al.[18] The method included the following steps (Fig. 1):

1. Translation

1.1 Preparation and forward translation

Initially, OHIP-TMDs principal author was contacted by email. Professor Justin Durham Ph.D. (Head, School of Dental Sciences, Newcastle University, UK), who owns the copyright of OHIP-TMDs, was granted a license to translate and use the instrument. The original, English-language version of OHIP-TMDs was independently translated into Thai by the author (PR: T1) and occlusion and orofacial pain specialist (T2) residing in Thailand and fluent in English, resulting in two translated versions (OHIP-TMDs T1 and OHIP-TMDs T2).

1.2 Synthesis

Both versions were compared and revised by a reviewer who is an expert of occlusion and orofacial pain committees, resulting in the "OHIP-TMDs T12" version.

1.3 Back translation

The OHIP-TMDs T12 version was back-translated to English by a native English speaker (who works at Chulalongkorn university language institute) who was fluent in Thai and blinded to the original version and had no knowledge of the study. This step purposed to uncover conceptual translation problems and glaring contradictions from the preceding processes and to create a "back-translated" version (OHIP-TMDs BT12).

1.4 Review and approval of the questionnaire by the original author

The original author was given OHIP-TMDs BT12 version for revision. A couple of minor details of OHIP-TMDs BT12 were commented to the translation team (T1, T2, and expert committees) for additional correction. Then a revision meeting including T1, T2, and the expert committees was conducted to create a revised OHIP-TMDs T12, based on the original questionnaire, the original forward translation (OHIP-TMDs T12), and the back-translated version (OHIP-TMDs BT12).

A revised OHIP-TMDs T12 version was sent to native English speaker to create a revised OHIP-TMDs BT12 version. Finally, the revised OHIP-TMDs BT12 version was submitted to the original author for approval. When the back-translation text (revised OHIP-TMDs BT12) was fully approved, a revised OHIP-TMDs T12 (Thai text) can represent the "Thai OHIP-TMDs Prefinal Version". The prefinal instrument was used for pretesting with a targeted population (TMD patients).

2. Pilot testing (pretesting)

In this step, the target population's acceptance, comprehension, and clarity of the translated questionnaire were evaluated. We produced chairside interviews and reviewed the questionnaire with TMD patients to pinpoint troublesome queries for final questionnaire editing.

Pretesting was carried out at the TMJ and Orofacial Pain Clinic at Dental Hospital, Faculty of Dentistry, Chulalongkorn University. The inclusion criteria included TMD patients who: were age 18 years or older, were diagnosed by DC/TMD Axis I criteria,[19] had TMD symptoms more than three months, and can speak and read Thai fluently.

Patients were invited to participate in the study during their dentist appointment, and thirty patients who accepted were included in the study. Participants were asked to fill in the demographic data and complete the questionnaire. Then, semi-structured interviews were individually performed with participants by the author (PR) to clarify and collect opinions or suggestions on each instrument's item from participants. The data from the questionnaire was used to calculate the internal reliability of the instrument using Cronbach's alpha coefficient.

3. Data analysis

Analysis of the results of pretesting and conclusion

Data analysis was performed with IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA). Descriptive statistics were reported as mean and standard deviation for continuous variables or frequency and percentage for categorical variables. Cronbach's alpha coefficients were used to evaluate internal consistency of the multiple-question Likert scale instrument. A score of more than 0.90 was suggest as an excellent internal consistency.[20] Mann-Whitney U test was used to examine the significant differences of OHIP-TMDs score between male and female participants. The correlation between two variables were calculated with Spearman Correlation (p<0.05).

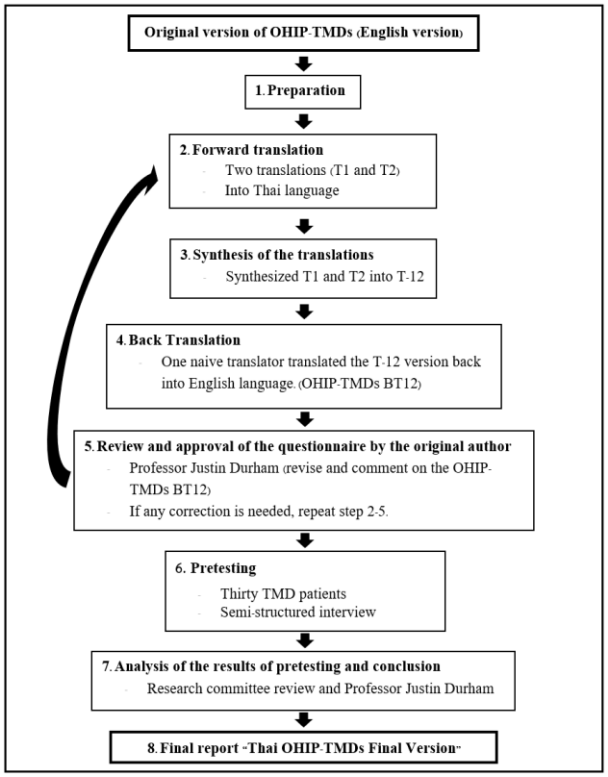

Figure 1. Flowchart of the translation and cross-cultural adaptation of the Thai version of OHIP-TMDs.

Results

The details of each domain of the original version of OHIP-TMDs were described in Table 1. From the translation and cross-adaptation process, the reviewer stressed the importance of maintaining the equivalence and examined each item separately in an effort to preserve the features from the original instrument,[18] resulting in "OHIP-TMDs T12" version. Following the revision of questionnaire by the original author, minor details of OHIP-TMDs

Table 1. The component and definition of each PROs of OHIP-TMDs.

Domains	Meaning[21,22]	The Question of OHIP-TMDs*
Functional limitation	Impairment results in restrictions or inability to conduct action or activity in a manner or within the range that is regarded as normal.	**Question 1:** Have you had difficulty chewing any foods because of problems with your jaws, teeth, or mouth? **Question 2:** Have you had difficulties opening or closing your mouth?
Physical pain	People claim that something hurts their bodies and is associated with bodily tissue damage.	**Question 3:** Have you had painful aching in your mouth, face or ear? **Question 4:** Have you had a sore jaw? **Question 5:** Have you had headaches because of problems with your jaws, teeth, or mouth? **Question 6:** Have you found it uncomfortable to eat any foods because of problems with your jaws, teeth, or mouth? **Question 7:** Have you felt that talking was painful because of problems with your jaws, teeth or mouth?
Psychological discomfort	A condition of emotional distress brought on by pressures and demands that are challenging to handle in daily life.	**Question 8:** Have you been worried by jaw or dental problems? **Question 9:** Have you been self conscious because of your jaws, teeth, or mouth? **Question 10:** Have jaw or dental problems made you miserable? **Question 11:** Have you felt tense because of problems with your jaws, teeth, or mouth?
Physical disability	Outcome of impairment in terms of functional performance and activity associated with physical depends on the person.	**Question 12:** Have you had to avoid eating some foods because of problems with your jaws, teeth, or mouth? **Question 13:** Have you had to interrupt meals because of problems with your jaws, teeth, or mouth?
Psychological disability	Outcome of impairment in terms of functional performance and activity associated with mental health depends on the person.	**Question 14:** Has your sleep been interrupted because of problems with your jaws, teeth, or mouth? **Question 15:** Have you been upset because of problems with your jaws, teeth, or mouth? **Question 16:** Have you found it difficult to relax because of problems with your jaws, teeth or mouth? **Question 17:** Have you felt depressed because of problems with your jaws, teeth, or mouth? **Question 18:** Has your concentration been affected because of problems with your jaws, teeth, or mouth?
Social disability	Outcome of impairment in terms of functional performance and activity associated with society depends on the person.	**Question 19:** Have you been a bit irritable with other people because of problems with your jaws, teeth, or mouth? **Question 20:** Have you had difficulty doing your usual jobs because of problems with your jaws, teeth, or mouth?
Handicap	Disadvantages that the person has because of their limitations and disabilities.	**Question 21:** Have you felt that life in general was less satisfying because of problems with your jaws, teeth, or mouth? **Question 22:** Have you been unable to work to your full capacity because of problems with your jaws, teeth, or mouth?

*All questions phrased as "Over the last month…".

BT12 were commented. The translation team had repeated the forward translation and back translation process and then resulted in a "Thai OHIP-TMDs Prefinal Version" that was used for pretesting in Thai TMD patients.

The pretesting took place at the Occlusion and Orofacial Pain Clinic, Faculty of Dentistry, Chulalongkorn University. Interviews were conducted in twenty-three women and seven men who met the research inclusion criteria. The pilot participants have a mean age of 33.93 ± 12.23 years. All recruited subjects completed the questionnaires and were interviewed to discuss on each questionnaire component.

From the pretesting, the demographic characteristics of participants are shown in Table 2, and OHIP-TMDs scores are summarized in Table 3. The recruited participants had broad characteristics, including wide range of age, TMD diagnosis, and TMD symptoms duration. When calculated the OHIP-TMDs scores, participants also had wide scores range, from three to seventy-six.

During the pretest, the interviewer collected the participants' suggestion on wording modification for more clarity and easier understanding. There are none remarkable changes required on each question. A final meeting of the translation committee was conducted, and then resulted in the "Thai OHIP-TMDs Final Version", that was authorized from the original questionnaire's main author.

The total Cronbach's alpha coefficients of the Thai OHIP-TMDs was 0.961, displaying an acceptable internal consistency of the instrument. Values above 0.90 are regarded as suitable for an applied study, demonstrating both the reliability of the data and the instrument has excellent quality for interpretation.[20] In addition, none of the question had negative impact on internal consistency of the instrument, and all of the question were suitable to include in the instrument.

The results from pilot testing indicated that OHIP-TMDs' scores were comparable between male and female. Then we analyzed the correlation between participant's age and OHIP-TMDs score to investigate the influence of patient's factor on impact of disease. Age and question 2 and 3 had negative correlation (r=-0.503, *p*=0.005; r=-0.381, *p*=0.038; respectively). However, the overall score of OHIP-TMDs did not show significant correlation with age. When we considered each domain, we found that functional limitation domain was negative correlated with participants' age (r=-0.470, *p*=0.009). Overall, none of domains were associated with age. The details of correlation analysis were described in Table 4 and 5.

Discussion

This study showed that the process of translation and cultural adaptation of OHIP-TMDs resulted in an instrument with acceptable internal reliability. Additionally, the information collected from pilot testing of the instrument indicated disease's impact on quality of life, determining by the frequency of each question. The participants of this study were predominantly women, but we found that the OHIP-TMDs outcomes were not significantly different between genders. However, there were a negative correlation between participant's age and the functional limitation domain. Interestingly, we found that older TMD patients had less scores on functional limitation, pain in their mouth, face or ear and difficulty in opening or closing their mouth, compared to the younger ones. In contrary, results from another study measuring OHIP-TMD score showed that matured adult TMD patients presented higher scores of physical pain domain compared to younger ones.[23] However, our study interpreted the pilot testing in thirty TMD patients that was a small group, further validation process will include more participants.

The major limitation of the OHIP-TMDs is lack of interpretation guidelines. Even though our results showed that patients were affected from TMD in many aspects of life, we could not categorize the level of negative effect based on the instrument's score. This limitation causes difficulty in clinical use of the OHIP-TMDs. Some studies reported OHIP-TMDs responsiveness to TMD symptoms was similar to OHIP-49, providing the ability of the instrument to detect a clinically important change after treatment. The minimal important clinical difference (MICD) is the smallest score difference that patients perceive as either positive or as a worsening of their illness. MCID may help determining the cut point of PROMs, when link to the clinical changes between before and after treatment. The study from Yule et al.[14] showed MCID determined with OHIP-TMDs is at least 6.9, this was a mean change score of instruments by global transition judgement scale that patients rated "improved a little" score, which

Table 2. Characteristics of the participants in the pretesting (n = 30).

Variables	
Gender, n (%)	
Male	7 (23.33%)
Female	23 (76.67%)
Age (years), mean ± SD (range)	33.93 ± 12.23 (18-68)
Symptoms duration (months), mean ± SD (range)	23.77 ± 24.81 (3-84)
Diagnosis, n (%)	
Local myalgia	23 (76.67%)
Myofascial pain	6 (20%)
Arthralgia	13 (43.33%)
Disc displacement with reduction	19 (63.33%)
Disc displacement with reduction with intermittent locking	6 (20%)
Disc displacement without reduction	1 (3.33%)

Table 3. The OHIP-TMDs scores from pilot testing.

OHIP-TMDs Scores	Mean	SD	Minimum	Maximum
All participants	33.83	21.74	3	76
Male participants	32.86	14.80	12	48
Female participants	34.13	23.72	3	76

Table 4. Correlation between scores of each question of OHIP-TMDs and age.

The Question of OHIP-TMDs	Rho (r)	*p*-value
Question 1	-0.339	0.067
Question 2	-0.503	0.005*
Question 3	-0.381	0.038*
Question 4	-0.356	0.053
Question 5	-0.027	0.888
Question 6	-0.338	0.068
Question 7	-0.106	0.577
Question 8	-0.204	0.281
Question 9	-0.144	0.449
Question 10	-0.147	0.440
Question 11	-0.033	0.864
Question 12	-0.081	0.670
Question 13	-0.132	0.488
Question 14	0.064	0.738
Question 15	-0.259	0.167
Question 16	0.006	0.977
Question 17	-0.010	0.959
Question 18	-0.044	0.817
Question 19	-0.064	0.735
Question 20	-0.206	0.275
Question 21	-0.165	0.383
Question 22	-0.206	0.274

*Statistically significant ($p<0.05$).

Table 5. Correlation between scores of each domain of OHIP-TMDs and age.

Domains	Rho (r)	*p*-value
Functional limitation	-0.470	0.009*
Physical pain	-0.318	0.086
Psychological discomfort	-0.174	0.357
Physical disability	-0.106	0.575
Psychological disability	-0.067	0.725
Social disability	-0.175	0.355
Handicap	-0.213	0.258

*Statistically significant ($p<0.05$).

may infer in further clinical research. There were studies that used OHIP-TMDs to detect the clinical efficacy of the treatment.[24,25] OHIP-TMDs has also been used for assessing the relationship between quality of life and TMD severity,[26] and between TMD chronicity and oral health-related quality of life.[16]

We provided the first translation and cross-adaption of the OHIP-TMDs instrument among the adult population in Thailand. For future direction, psychometric testing, reliability, validity, as well as confirmatory factor analysis for the Thai version of OHIP-TMDs will be tested in Thai TMD patients. Additionally, the interpretation of OHIP-TMDs score will be validated with clinical changes to improve clinical uses of PROMs.

Conclusion

The OHIP-TMDs has been successfully translated and cross-culturally adapted into Thai. This instrument will allow its patient-centered outcomes measurement approach to evaluate the quality of life of TMD patients in Thailand. The future direction of the Thai version of OHIP-TMDs instrument includes psychometric testing, reliability, psychometric testing, reliability, validity, as well as confirmatory factor analysis and clinical application in an academic setting.

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Effects of Calcium Carbonate Microcapsules on Property of the Thermosensitive Hydrogel

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ABSTRACT

Background and Objective: To address a demand for an easy to use and bioactive bone substitutes and a necessity to improve control release property of the hydrogels, the current study aimed to fabricate the thermosensitive calcium carbonate microcapsule ($CaCO_3$)-nano hydroxyapatite (nHA)-chitosan/collagen hydrogels and investigate effects of the $CaCO_3$ on the property of the hydrogels.

Methods: The $CaCO_3$ microcapsules were prepared and characterized. Then the 1% and 2% (w/v) $CaCO_3$ were added in the 2% (w/v) nHA-10% (w/v) beta-glycerophosphate-chitosan/collagen hydrogel ($CaCO_3$-nHA-chitosan/collagen hydrogel) and then the sol-gel transition of the hydrogels was stimulated by the temperature change at 37ºC. Later, the effects of the $CaCO_3$ on microstructure and mechanical and physical strengths of hydrogels and a cell cytotoxicity test were investigated (n = 3-5, Mean ± SD).

Results: The average porosity of the 0%, 1% and 2% $CaCO_3$-nHA-chitosan/collagen hydrogels was 82.53 ± 4.99% and the 2% $CaCO_3$ improved a well-defined porous structure of the hydrogel with pore size 128.07 ± 34.81 µm. The incorporation of the 1% and 2% $CaCO_3$ microcapsules in the 2% nHA-hydrogel significantly increased mechanical and physical strengths of the hydrogels ($p<0.05$). The 1% and 2% $CaCO_3$-nHA-chitosan/collagen hydrogels exhibited averages low compressive strength at 4,974.83 ± 354.34 Pa, high swelling degrees at 214.08 ± 20.97%, and low degradation rates at 15.24 ± 5.83% and were non-cytotoxic.

Conclusion: The $CaCO_3$ microcapsules improved porous structure and morphological stability of the $CaCO_3$-nHA-chitosan/collagen hydrogel. Based on the microstructure and optimal pore size, the 2% $CaCO_3$-2% nHA-hydrogel exhibited favorable characteristics for promoting bone regeneration.

Keywords: Bone regeneration, Calcium carbonate microcapsules, Cell cytotoxicity, Hydroxyapatite nanoparticles, Thermosensitive chitosan/collagen hydrogel

Proceeding

Introduction

The incidence of tooth loss and a greater need for dental implant and prosthesis are heighten in the aging society.[1] At the same time, alveolar bone resorption following tooth loss necessitates an alveolar bone augmentation to create sufficient volume and dimension of the alveolar bone to sustain form and function of the prostheses.[2] Bone substitutes have played important roles in reducing the use of autologous bone, improving bone augmentation outcomes, and increasing patient satisfaction.[3]

Bone substitutes should be bioactive and could function as a delivery vehicle for bioactive proteins or molecules such as bone morphogenetic protein-2 (BMP-2).[4] The thermosensitive hydrogel system provides several advantages because it is easy to use and able to fill in the defects with irregular shapes and limited access while causing minimal injury to the patients.[5] The hydrophilic environment and high porosity of the hydrogel system facilitate cell infiltration, formation of blood vessels, and subsequent bone regeneration.[6] It has been reported that the chitosan/collagen composite matrix promotes growth and osteogenic differentiation of bone marrow stromal cells.[7,8] An ability of hydroxyapatite (HA) to support osteoblast adhesion and proliferation in vitro and to establish a strong bonding with the newly deposited bone mineral have been clearly demonstrated.[9] Besides, inorganic matrixes of the HA could support control release function of the hydrogel by increasing protein absorption and then gradually released the osteogenic protein (BMP-2).[10] Calcium carbonate microcapsule ($CaCO_3$) exhibits an excellent biocompatibility and have been used for biomedical applications such as bone regeneration and drug delivery.[11] Bioactive molecules are absorbed on the surface of the porous $CaCO_3$ and the hollow microcapsules could be applied for different drug deliveries.[12] Therefore, the current study aimed to fabricate the thermosensitive $CaCO_3$-nHA-chitosan/collagen hydrogel and investigate effects of the $CaCO_3$ on property and cytotoxicity of the hydrogels.

Materials and Methods

Preparation of the Calcium Carbonate Microcapsules

Calcium carbonate microcapsules ($CaCO_3$) were prepared by precipitating sodium carbonate with calcium chloride in the carboxymethyl cellulose at room temperature (RT). An equal volume of the 0.33 M sodium carbonate solution was poured into the 1.0 g/L carboxymethyl cellulose under stirring, and then at 40ºC under a

rapid stirring an equal volume of the 0.33 M calcium chloride solution was added into the mixture. After that, the mixture was left to stand at RT for 30 min and then centrifuged at 3,500 rpm for 5 min. Then the white slurry was washed with DI water and ethyl alcohol, respectively for 2 times each, kept at -80°C and freeze-dried (All chemical were from Sigma-Aldrich, St Louis, MO).[13]

Preparation of a Thermosensitive Nano Hydroxyapatite-Calcium Carbonate Microcapsule-Chitosan/Collagen Hydrogel

The thermosensitive calcium carbonate microcapsule ($CaCO_3$)-nano hydroxyapatite (nHA)-4:1 chitosan/collagen hydrogels were prepared according to previous studies.[5,14,15] In brief, the 4:1 (w/w) or 2:1 (v/v) chitosan-collagen composite hydrogel were prepared by adding the 1% atelocollagen (Koken, Tokyo, Japan) into the 2% chitosan in 0.1 M acetic acid in a ratio of 2:1 (v/v) under stirring on ice. After that the mixture of the ice cold 56% (w/v) beta-glycerophosphate (bGP), nHA and $CaCO_3$ were dropwise added into the chitosan/collagen matrix under stirring to achieve the 0%, 1% and 2% (w/v) $CaCO_3$–2% (w/v) nHA-10%(w/v) bGP-4:1(w/w) chitosan/collagen hydrogels, according to groups of study (All chemical from Sigma-Aldrich). Then, the hydrogels were loaded in the Teflon molds and containers for further investigations in solution and gel stages. For the hydrogel in gel stage, the hydrogels were incubated in the 37ºC incubator for 18 h before testing.[5,15]

The Levels of pH and Setting Time Determination

Levels of the pH of the hydrogels in a solution stage on ice were measured (Orion 2 star, Thermo Fisher Scientific, Waltham, MA). After that, the hydrogels were incubated at 37°C and a setting time was recorded when the mobility was not seen and the hydrogels were changed from clearly liquid to the milky white gel.[5,15]

Microstructures Analysis

The microstructures of the hydrogels and micro capsules were examined using a scanning electron microscope (SEM) (JSM 7800F, JOEL, Tokyo, Japan). The freeze-dried hydrogel disks, size 5x3 mm and the $CaCO_3$ microcapsules were examined under the scanning electron microscope (SEM) (JSM 7800F, JOEL, Tokyo, Japan). Then, the porous structure and distribution of the nHA and $CaCO_3$ in the hydrogels, and size and shape of the microcapsules were examined. Later, sizes of the microcapsules and pore size of the hydrogels were measured by drawing the straight line across the widest part of particles and pores on the SEM images at ×2,000 and × 100 magnifications, respectively using the Image J program (ImageJ 1.×, National Institutes of Health, Bethesda, Maryland).[15]

Porosity Analysis

The porous structures of the 5x3 mm freeze-dried hydrogel disks, 3 disks per sample were examined using a mercury intrusion porosimeter (MIP) (Auto pore V, Micrometrics, Norcross, Georgia).[15]

Mechanical Property Test

Compressive strength of the hydrogel was tested using a texture analyzer testing machine (TA-XT2i, Stable Micro System, Surrey, UK) in an unconfined condition. The 20x5 mm hydrogel disks were placed under a Delrin platen and then a small tare load of 0.01 N was applied at a ramp speed at 20% strain per second until reaching the 10% strain. Then, the compressive stress values were recorded.[15]

Swelling Test

The swelling ratio was utilized to quantify the water absorption capacity of the hydrogels. The 5x3 mm freeze-dried hydrogel disks were weighted before being immersed in the phosphate buffer saline (PBS) (W_0) and then incubated in the PBS at 37°C for 1, 3, 24, 48, and 72 h. Then, at each investigation time, the hydrogels were picked up by forceps and hanged in air for 1 minute and briefly touched on the absorbent paper to remove the excess fluid (Whatman filter paper Grade 4, GE Lifesciences, Issaquah, WA) before weighting (W_1). After that the percentages of the swelling ratios were calculated as follows: Percentages of swelling ratio = $(W_1-W_0)/(W_0) \times 100$.[7]

Degradation Test

The 5x3 mm freeze-dried hydrophilized hydrogel disks were incubated in the PBS overnight to neutralize the hydrogels and weighted (W_0) (as described in the Swelling test). Then, the hydrogels were incubated in the 10,000 U/L lysozyme (Chicken lysozyme, Sigma-Aldrich) for 28 days at 37ºC. After that on days 3, 5, 7, 14, 24 and 28, the hydrogels were collected, washed in DI water, and kept at -80°C for freeze-drying and weighting (W_1). Then, the percentages of weight loss were calculated as follows: Percentages of weight loss = $(W_0-W_1)/(W_0) \times 100$.[7]

Cytotoxicity Test

Cell cytotoxicity of the 2% $CaCO_3$-2% nHA-chitosan/collagen hydrogel and the 2% nHA-chitosan/collagen hydrogel alone were tested with the human fetal osteoblast cell line (hFOB1.19, ATCC, Manassas, Virginia). The hydrogels were added into a 6 well cell culture plate, 2 ml/well and incubated in an incubator at 37°C with 5% CO_2 and 95% humidity for 18 h. After that the hydrogels were washed in DI water and then incubated in growth medium, 2 ml/well in an incubator for 7 days. Then on days 1, 2, 3, and 7, the incubated culture mediums were collected, kept at -20°C and replaced with a new medium.

After that the hFOB1.19 cell line at passage 5 were seeded in a 96 well cell culture plate at $3x10^3$ cells/well and cultured in a growth medium for 24 h. Then, the cell culture medium was changed to the pre-incubated culture mediums according to groups of the study and cultured for 72 h. A growth medium comprised phenol red free DMEM-F12 and 10% fetal bovine serum (FBS), 1% Penicillin streptomycin and 0.05% Fungizone (all from Gibco, Thermo Fisher Scientific, Waltham, MA). Then, cell viability assay was performed using a CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI) following the manufacturing's instruction, in which the optical density (OD) was measured at 490 nm using a microplate reader (Varioskan Flash, Thermo Fisher Scientific). Subsequently, the percentages of cell viability were calculated relatively to the OD values of a control group in a regular growth medium, as follows: Percentages of cell viability = (OD of sample/OD of control) x 100.[14]

Statistical Analysis

Numbers of samples at each investigation time were 3-5 samples. Data was reported as Mean ± SD. Statistical analysis was performed using GraphPad Prism Version 9.0 on a personal computer. Data were described and tested for normality using Kolmogorov-Smirnov test. Subsequently, if the distribution was normal, the data were compared between groups and between different investigation times using the One-way ANOVA and Repeated measures ANOVA, respectively. When the distribution of the data was not normal, the Kruskal-Wallis's test for independent samples were employed for the comparisons between groups, and the Friedman test for matched samples were employed for the comparisons between times. Then the Turkey's and Dunn-Bonferroni post hoc tests, respectively were used for the multiple comparisons. Significant difference was set at $p<0.05$.

Results

The Calcium Carbonate Microcapsules Increased pH levels of The Hydrogels to a Physiologic Level and Decreased Setting Time

The pH of the chitosan-collagen composite hydrogel was mild acidic at pH 7.05. When the 2% nHA was added in the chitosan/collagen hydrogel, the pH of the nHA-chitosan/collagen hydrogel was increased to 7.52. Further, when the 1% and 2% $CaCO_3$

microcapsules were added in the 2% nHA-chitosan/collagen hydrogel the pH values were 7.49 and 7.66, and the setting time were 15 min and 10 min, respectively.

The Thermosensitive Calcium Carbonate-Nano Hydroxyapatite Chitosan/Collagen Hydrogels Were Porous, and the Calcium Carbonate Microcapsules Improved Porous Architecture of the Hydrogels

The SEM analysis demonstrated a porous structure with an opened and interconnected pore architecture of the $CaCO_3$-nHA-chitosan/collagen hydrogels (Fig. 1A-C) and the round and porous morphology with rough surface of the $CaCO_3$ microcapsules. The average size of the microcapsules was 3.37 ± 0.51 μm (2.325 μm – 4.852 μm) (Fig. 1D). The well-defined porous structure of the hydrogels was enhanced with the increase of the concentrations of the $CaCO_3$ (Fig. 1C & E). The average pore size of the 2% $CaCO_3$-hydrogel was 128.07 ± 34.81 μm (Fig. 1E). The deposition and distribution of the fine particles of the nHA and large particles of the microcapsules could be seen on the pore wall of the hydrogels (Fig. 1F). The MIP analysis demonstrated that the porosity of the 0% - 2% $CaCO_3$-2% nHA hydrogels were higher than 80% of a total volume. The porosity of the 2% $CaCO_3$ hydrogel was 82.53 ± 4.99 % and tended to be higher than other groups (Fig. 1G).

The Calcium Carbonate Microcapsules Increased Mechanical Strength of the Hydrogels

A compressive stress test was performed to assess the material deformation in response to a compressive force. The 1% and 2% $CaCO_3$ microcapsules significantly increased compressive strength of the 2%nHA-chitosan/collagen hydrogels (p<0.05), but the strengths of the 1% and 2% $CaCO_3$ groups were not significant different (p>0.05). An average compressive strength of the 1% and 2% $CaCO_3$-hydrogels was 4,747 ± 100.70 Pa and 5,202.67 ± 341.17 Pa, respectively (Fig. 2).

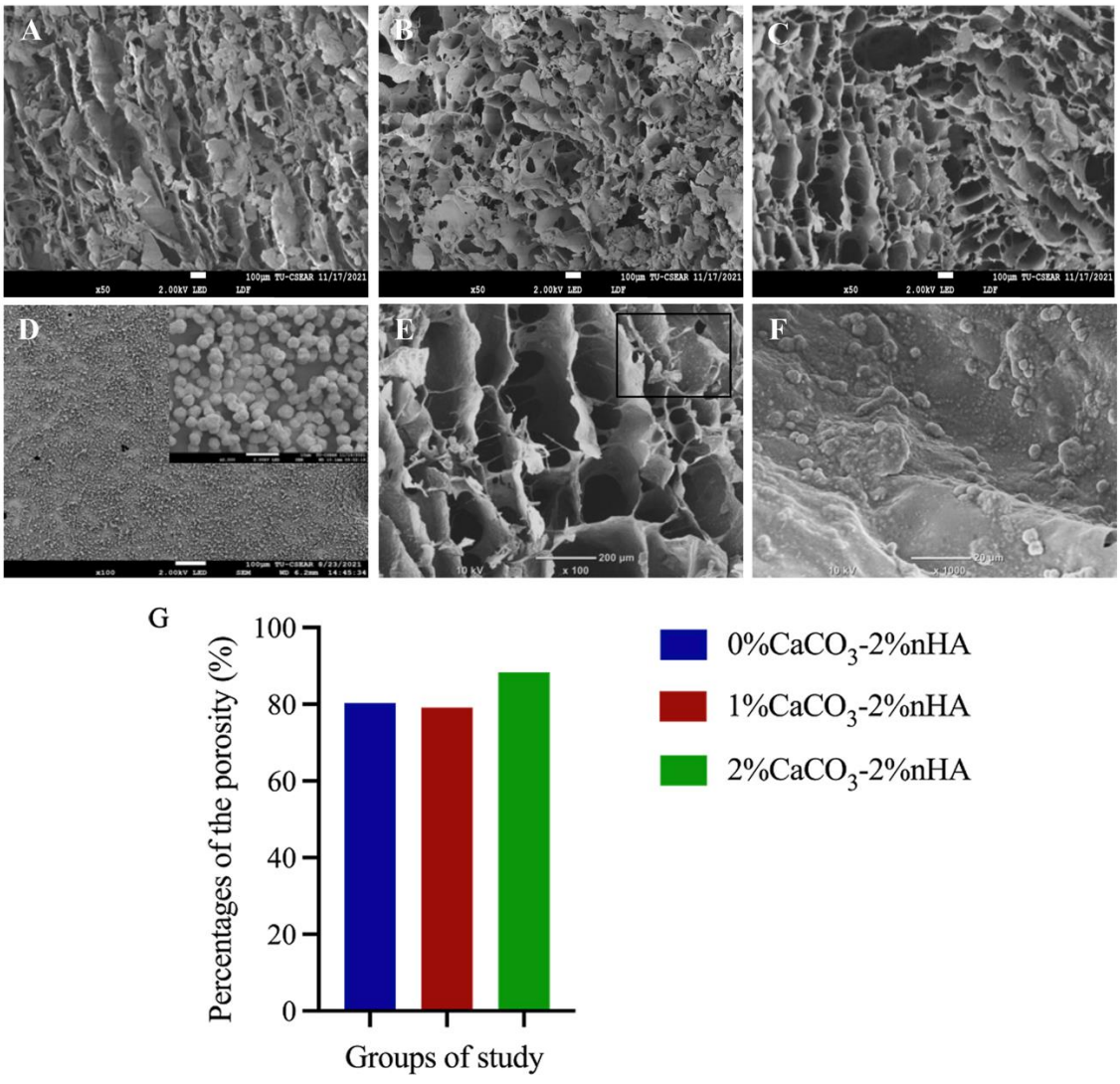

Figure 1. Demonstrating scanning electron microscopy (SEM) images and the mercury intrusion porosimetry (MIP) test of the 0% - 2% calcium carbonate microcapsules ($CaCO_3$) – 2% (w/v) nano hydroxyapatite (nHA) – 4:1 (w/w) chitosan/collagen hydrogels ($CaCO_3$-hydrogels), (A) 0%, (B) 1% and (C) 2% $CaCO_3$-hydrogels, (D) the $CaCO_3$ microcapsule, (E & F) the magnified images of the 2% $CaCO_3$ hydrogel, (F) a pore wall of the 2% $CaCO_3$-hydrogels exhibiting deposition of small and large particles of the nHA and $CaCO_3$, respectively and (G) The mercury intrusion porosimetry (MIP) test demonstrating a total porosity of the hydrogels.

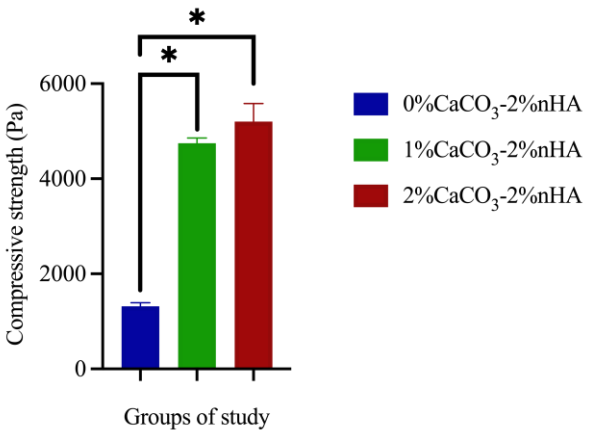

Figure 2. Exhibiting compressive strength of the the 0% - 2% calcium carbonate microcapsules ($CaCO_3$) – 2% (w/v) nano hydroxyapatite (nHA) – 4:1 (w/w) chitosan/collagen hydrogels ($CaCO_3$-hydrogels). Abbreviations 0% $CaCO_3$-2% nHA denote 0% $CaCO_3$-hydrogels, and 1% $CaCO_3$-2% nHA and 2% $CaCO_3$-2% nHA, the 1% and 2% $CaCO_3$-hydrogels, respectively. A symbol * represents a significant difference at $p<0.05$ (n=5, Mean ± SD).

The Calcium Carbonate Microcapsules Decreased Swelling Degree and Degradation Rate of The Hydrogels

Swelling Test

During the 72-h incubation, the percentages of weight gain of the 0% $CaCO_3$-2% nHA-chitosan/collagen hydrogel was gradually decreased, in which the percentages of weight loss at 1 h and 72 h incubation were significantly different ($p<0.05$), but the percentages of weight gain of the 1% and 2% $CaCO_3$-hydrogel were relatively stable. The percentages of weight gain of the 1% and 2% $CaCO_3$ groups at 1h and 72 h and between groups were not significantly different ($p>0.05$). At 1h – 24 h, the swelling rates of the 0% $CaCO_3$ group were significantly higher than the 1% and 2% $CaCO_3$ groups ($p<0.05$), in which the average swelling degrees at 24 h of the 0%, 1% and 2% $CaCO_3$ groups were 368.33 ± 44.54%, 230.75 ± 11.77%, 197.42 ± 12.51%, respectively. Then, at 48h and 72 h the swelling degrees of all groups were not significantly different with an average swelling degree of 279.57 ± 38.83% at 72 h (Fig. 3A).

Degradation Test

The degradation degrees of the hydrogels were gradually increased from days 1 to 28. Throughout the 28 days-incubation, the percentages of weight loss of the 0% $CaCO_3$-2% nHA-chitosan/collagen hydrogels were significantly higher than the 1% and 2% $CaCO_3$ groups ($p<0.05$), while the degradation rates of the 1% and 2% $CaCO_3$ groups were not significantly different ($p>0.05$). The percentages of weight loss of all group on day 28 were significantly higher than day 3 ($p<0.05$). On day 28, the average percentages of weight loss of the 0%, 1% and 2% $CaCO_3$-hydrogels were 33.99 ± 2.93%, 19.85 ± 3.21% and 10.62 ± 3.61%, respectively (Fig. 3B).

The Thermosensitive Calcium Carbonate-Nano Hydroxyapatite-Chitosan/Collagen Hydrogels were non-Cytotoxic and could Promote Cell Growth

The cell viability assay demonstrated that the 0% and 2% $CaCO_3$-2%nHA-chitosan/collagen hydrogels were non-cytotoxic. When the hFOB1.19 were cultured with the pre-incubated mediums for 72 h, it was found that an average of the percentages of cell viability of the hFOB1.19 cultured with the pre-incubated mediums for 72 h were 126.73 ± 5.91% relatively to the control in a regular growth medium. Furthermore, the percentages of cell viability of cells in the pre-incubation mediums, collected from the incubation-days 2, 3 and 7 were significantly higher than that was collected on day 1 ($p<0.05$). The differences between the 0% and 2% $CaCO_3$-hydrogels were not significantly different ($p>0.05$) (Fig. 4).

Discussion

Goals of the presents study were to develop an injectable and bioactive $CaCO_3$-nHA-chitosan/collagen hydrogels that could function as a delivery vehicle of biomolecules and stem cell for promoting bone regeneration. The study was designed based on previous reports on osteoconductive and control release properties of the thermosensitive chitosan/collagen hydrogels[14] and the advantages of incorporating beta-tricalcium phosphate[15] and nano hydroxyapatite in the thermosensitive hydrogels.[10] Therefore, the current study incorporated the beneficial elements which are 2% nHA to improve osteoconductive property and mechanical strength of the chitosan/collagen hydrogel and added $CaCO_3$ microcapsules to improve control release capacity of the nHA-hydrogel. It was expected that the hollow and porous structure of the $CaCO_3$

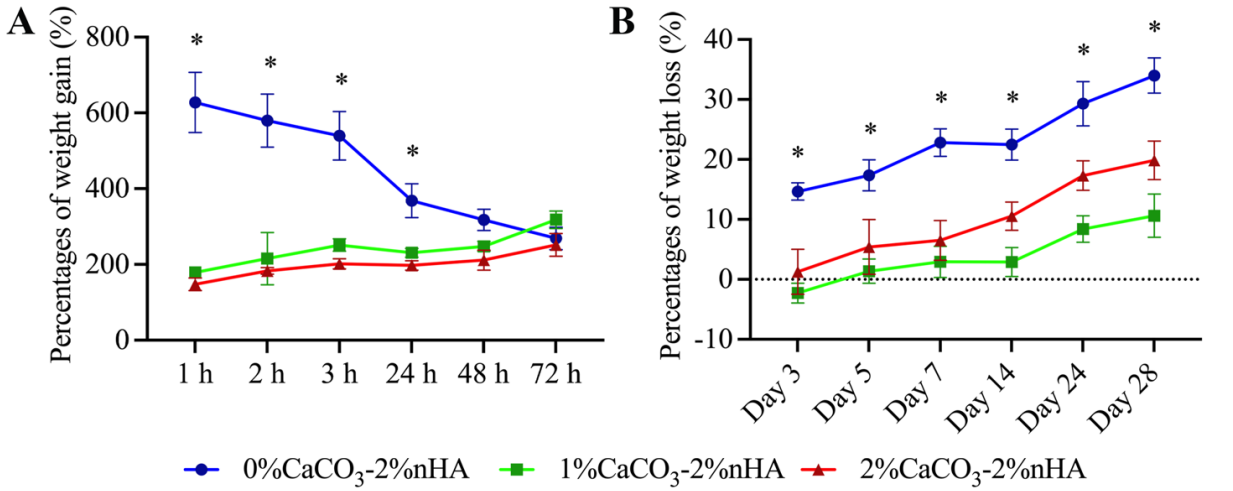

Figure 3. Demonstrating physical property of the 0% - 2% calcium carbonate microcapsules ($CaCO_3$) – 2% (w/v) nano hydroxyapatite (nHA) – 4:1 (w/w) chitosan/collagen hydrogels ($CaCO_3$-hydrogels). (A) Swelling degrees and (B) Degradation rates. Abbreviations 0%$CaCO_3$-2% nHA denote 0% $CaCO_3$-hydrogels, and 1% $CaCO_3$-2% nHA and 2% $CaCO_3$-2% nHA, the 1% and 2% $CaCO_3$-hydrogels, respectively. Symbols * represent significantly higher than the other groups at $p<0.05$ (n=5, Mean ± SD).

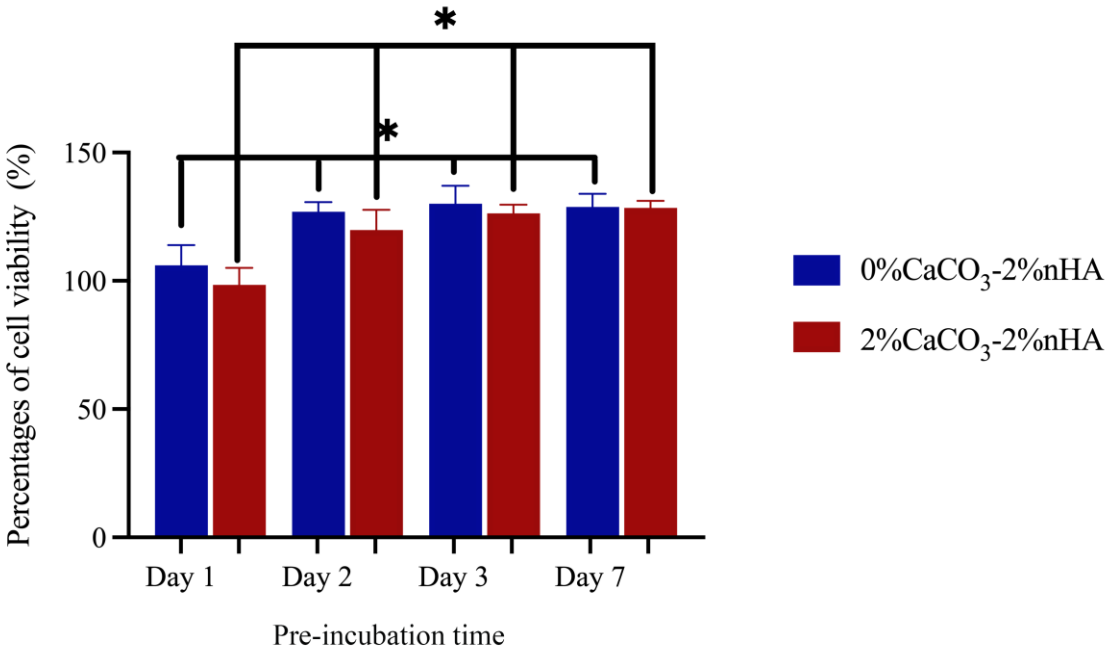

Figure 4. Demonstrating cell cytotoxicity of the 0% - 2% calcium carbonate microcapsules ($CaCO_3$) – 2% (w/v) nano hydroxyapatite (nHA) – 4:1 (w/w) chitosan/collagen hydrogels ($CaCO_3$-hydrogels) on cell viability of the human fetal osteoblast cell line (hFOB1.19). Abbreviations 0%$CaCO_3$-2% nHA denote 0% $CaCO_3$-hydrogels, and 1% $CaCO_3$-2% nHA and 2% $CaCO_3$-2% nHA, the 1% and 2% $CaCO_3$-hydrogels, respectively and Pre-incubation time, the time that culture mediums were pre-incubated with the particles. Symbols * represent significantly higher than the other groups at $p<0.05$ (n=5, Mean ± SD).

microcapsules would facilitate the storage of the biomolecules such as natural flavonoids or bone morphogenetic proteins in the chitosan/collagen composite matrix for a prolong and regulating release manner.[12,13]

The findings demonstrated that the microcapsules did not interfere with the gelation process of the $CaCO_3$-nHA-chitosan/collagen hydrogels and the $CaCO_3$ enhanced the well-defined porous structure of the 2% nHA-chitosan/collagen hydrogel. It could be hypothesized that the distribution of the microcapsules into the gaps between the nHA particles in the matrix of the hydrogel contributed to the increase of the mechanical strength and physical stability of the $CaCO_3$-nHA-hydrogels.[16] Additionally, the amount the $CaCO_3$ at the 1% and 2% (w/v) did not overload the capacity of the nHA-chitosan/collagen matrix to support the microcapsules in the hydrogels, as it was demonstrated by the distribution of the small and large particles of the nHA and microcapsules on the pore walls. Additionally, the $CaCO_3$ microcapsules could also be a source of calcium and nidus for the mineralization of the bone matrix.[11] Therefore, together with the osteoconductive property of the nHA that can enable integration of the newly formed bone with the host bone,[9] the $CaCO_3$-nHA-chitosan/collagen hydrogel is a bioactive scaffold that would enhance growth and osteoblastic differentiation of mesenchymal stem cells and bone regeneration.

Furthermore, as the microstructure of the hydrogel is a crucial factor regulating bone regeneration capacity of the tissue engineered constructs,[8,17] the opened and interconnected pore architectures of the 2% $CaCO_3$-2% nHA-chitosan/collagen hydrogel with a pore size larger than 100 µm would improve bone regeneration in the inner structure of the hydrogels.[5,7,8] The rough and strong pore walls could further support cell adhesion and matrix deposition on the porous structure of the hydrogel.[10,12] Additionally, capacity of the $CaCO_3$-nHA-chitosan/collagen hydrogels to support cell growth and function as delivery vehicles for bioactive molecules was clearly demonstrated by the degrees of swelling and degradability, physiologic pH levels and non-cytotoxicity of the hydrogels.[18-20] The findings on the degradation rates suggested that the structural stability and intermolecular bonding within the matrix of the 1% and 2% $CaCO_3$ were higher than the 0% $CaCO_3$-2% nHA-hydrogels.[15,21] Besides, the high levels of cell viability in groups with and without $CaCO_3$ suggested that the incorporating of the 1%-2% $CaCO_3$ did not adversely affect the non-cytotoxicity and ability to promote cell growth of the 2% nHA-chitosan/collagen hydrogel. However, even though the $CaCO_3$ increased mechanical strength of the $CaCO_3$-nHA hydrogels, the compressive strength of the hydrogel at 4,974.83 ± 354.34 Pa was still much lower than that of the cancellous bone at 0.1-16 MPa.[22] Therefore, the hydrogel must be applied in the well-defined 3 wall-defects with the supporting membrane.[23]

Conclusion

The 1% and 2% $CaCO_3$ microcapsules improved porous structure, increased mechanical strength and physical stability of the 2% nHA-chitosan/collagen hydrogels. Based on the porous structure and pore size, the 2%$CaCO_3$-2%nHA-chitosan/collagen hydrogels exhibited the favorable characteristics for promoting bone regeneration. Further studies would be conducted to examine the control release property and the biocompatibility of the hydrogel in an animal model.

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Microtensile Bond Strength of Resin-Resin Interfaces under Immediate Saliva Decontamination Methods

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ABSTRACT

Background and Objective: To investigate the influence of different saliva decontamination methods on microtensile bond strength (μTBS) between resin-resin interfaces.

Methods: Light-cured rectangular resin composite (Filtek™ Z350XT shade A4) blocks were randomly divided into nine groups; G1 (controlled) was not contaminated, G2-G9 were contaminated with saliva followed by various decontamination methods. G2 air drying; G3 rinsing and drying; G4 rinsing, drying, and etching with 37% phosphoric acid; G5 drying, and application of Clearfil™ SE Bond; G6 rinsing, drying, and application of Clearfil™ SE Bond; G7 drying, application of Clearfil S3 Bond Universal; G8 rinsing, drying, and application of Clearfil S3 Bond Universal and G9 rinsing and drying, etching with 37% phosphoric acid, application of Clearfil S3 Bond Universal. After the decontamination process, specimens were bonded with resin composite shade A1. After water storage, all blocks were sectioned into beam configuration with a cross-sectional area of approximately 1 mm^2 (N=15). Microtensile bond strength tests were analyzed using the one-way ANOVA and followed by Dunnett's T3 multiple comparison tests (p<0.05).

Results: G1 showed a significantly higher μTBS than the other groups that were contaminated by saliva. Among the contaminated saliva groups, the group which was decontaminated by etching with 37% phosphoric acid (G3) provided the highest μTBS while the μTBS of all Clearfil™ SE Bond and Clearfil™ S3 Bond Universal (G5-G9) showed lesser μTBS than the groups that were decontaminated without using adhesive.

Conclusion: The saliva decontamination process through rinsing, drying, and etching with 37% phosphoric acid provided the highest microtensile bond strength.

Keywords: Resin composite, Resin-resin interface, Saliva decontamination

Proceeding

Introduction

Resin composite is now widely used in dental restoration because it provides high esthetic, as well as being consistent with the principles of minimally invasive dentistry.[1] However, conventional resin composite exhibit shrinkage properties during light cure polymerization, wherein filling the whole cavity with one piece of resin composite can lead to the possibility of failure due to the polymerization shrinkage. Therefore, resin composites requires incremental restorative methods to reduce the configuration factor.[2]

Each step of the resin composite restoration requires moisture control to achieve long-term success.[3] Restoration under a rubber dam is a standard treatment to prevent injury to the oral organs and help control humidity from the oral cavity during the restorative process. In cases where rubber dam cannot be used, saliva contamination is a common problem in restorative procedure.[4] The contamination can occur in several steps which can reduce the bond strength of resin-resin interfaces, and decrease a long-term success.[5]

Most of the researches that involve saliva contamination to resin composite filling focus on the effect of contaminated saliva and decontamination methods that occur during the adhesive process[6-9] However, the few existing studies on resin-resin interfaces and the effect of the decontamination are unclear. Hence, this research intended to investigate various decontamination methods to identify the appropriate treatment to use in future clinical applications.

This study aimed to investigate the influence of different saliva decontamination methods on microtensile bond strength between resin-resin interfaces.status in a non-invasive way, it will be beneficial for the medical field.

Materials and Methods

This study was approved by the Human Experimentation Committee, Faculty of Dentistry, Chiang Mai University (14/2021). Twenty-seven resin composite blocks were made by filling resin composite (Filtek™ Z350 XT A4, 3M ESPE, MN, USA) in a 1.5x10x6 mm^3 split mold with incremental technique, as shown in Fig. 1. Each increment was added to a thickness of 1.5 mm and light cured for 20 seconds at the light intensity of 1,200 mW/cm^2 (Bluephase™ LED curing light, Ivoclar Vivadent, Schaan, Liechtenstein). On the last layer, a spatula was used to produce a flat surface to retain and replicate the oxygen inhibited layer in the clinical situation.

The specimens were randomly divided into nine groups. Group 1 (G1) contained composite blocks without saliva contamination or any decontamination methods was used as a controlled group. The rest of the specimen's group (group: G2-G9) were applied with the saliva. The saliva was collected from

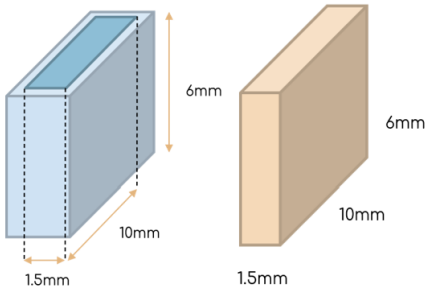

Figure 1. Split mold and resin composite block.

a single individual at the site and at the same time. The resin composite blocks were made, and saliva was applied to the composite blocks with a microbrush for 10 seconds.

Eight different decontamination methods were used for each group as follow:

Group 2 (G2) – air drying for 20 seconds.

Group 3 (G3) – rinsing with water for 20 seconds and drying.

Group 4 (G4) – rinsing, drying, etching with 37% phosphoric acid for 15 seconds, rinsing and drying.

Group 5 (G5) – drying and application of the adhesive (Clearfil™ SE Bond, Kuraray Medical, Tokyo, Japan) according to the manufacturer's instructions.

Group 6 (G6) – rinsing, drying, and application of Clearfil™ SE Bond.

Group 7 (G7) – drying and application of the adhesive (Clearfil™ S3 Bond Universal, Kuraray Medical, Tokyo, Japan) according to the manufacturer's instructions.

Group 8 (G8) – rinsing, drying, and application of Clearfil™ S3 Bond Universal.

Group 9 (G9) – rinsing, drying, etching with 37% phosphoric acid for 15 seconds, rinsing and drying, and application of Clearfil™ S3 Bond Universal.

The materials used in this study are shown in Table 1.

Each composite block was put into a 1.5x10x12 mm^3 metal split mold. Then resin composite shade A1 was added on top of the composite blocks by incremental technique. Afterward, the resin composite blocks were stored in distilled water at 37℃ for 24 hours.

The resin composite blocks were sectioned into a stick shape using a diamond saw (Isomet™ Low speed, Buehler Co., IL, USA) of approximately 1 mm^2 at a cross-sectional area. Each resin composite block could be sectioned into 5 specimens for a total of 135 specimens (n=15), as shown in Fig. 2. Specimens were stored in distilled water at 37℃ for 24 hours.

Each specimen was attached to a custom testing device with cyanoacrylate glue (Model repair II blue, Dentsply-Sankin, Otahara, Japan). Microtensile bond strength (μTBS) was tested by a universal testing machine (Instron 5566, Instron (Thailand) Limited, Thailand). The data were analyzed using one-way ANOVA and Dunnett's T3 (p<0.05), and the fracture areas were observed by a Scanning Electron Microscope; SEM (JSM-6610LV SEM, JOEL Ltd., Japan).

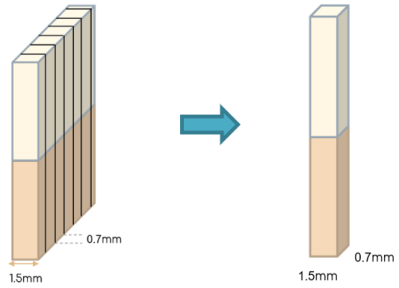

Figure 2. The resin composite blocks were sectioned into a stick shape.

Results

The mean microtensile bond strength is presented in Table 2. The homogeneities of the variance test showed significant differences between decontamination methods.

Table 1. Materials used in this study.

Product Names and Manufacture	Composition	Batch No.
Filtex™ Z350XT (3M ESPE, MN, USA)	Matrix: Bis-GMA, UDMA, Bis-EMA, TEGDMA Filler: SiO_2 nanofiller, ZrO_2 nanofiller, ZrO_2/SiO_2 nanocluster	A1: NC79377 A4: NC73322
Total Etch™ Etching Gel (Ivoclar Vivadent, Schaan, Liechtenstein)	37% Phosphoric acid	Z012CT
Clearfil™ SE Bond (Kuraray Medical, Tokyo, Japan)	Primer: 10-MDP, HEMA, Hydrophilic dimethacrylate, dl-Camphorquinone, N,N-Diethanol-p-toluidine, water Bonding agent: 10-MDP, BIS-GMA, HEMA, Hydrophobic aliphatic demethacrylate, dl-Camphorquinone, N,N-Diethanol-p-toluidine, colloidal silica	Primer: 1SO370 Bond: 1R0687
Clearfil™ S^3 Bond Universal (Kuraray Medical, Tokyo, Japan)	10-MDP, Bis-GMA, HEMA, Hydrophobic aliphatic dimethacrylate, dl-Camphorquinone, ethanol, water, Silane coupling agent, colloidal silica	280048

Table 2. Means and standard deviations of the microtensile bond strength data of the studied groups (mean ± SD, MPa).

Group	Saliva	Decontamination		Microtensile Bond Strength (mean ± SD)
		Surface Treatment	Adhesive	
1	-	-	-	$36.35^{A} \pm 2.31$
2	+	Drying	-	$25.25^{C} \pm 3.67$
3	+	Rinsing	-	$23.15^{C} \pm 2.69$
4	+	37% Phosphoric acid	-	$31.02^{B} \pm 3.71$
5	+	Drying	Clearfil™ SE Bond	$16.88^{D} \pm 3.75$
6	+	Rinsing		$16.37^{D} \pm 2.75$
7	+	Drying	Clearfil™ S3 Bond Universal	$15.90^{D} \pm 1.85$
8	+	Rinsing		$9.09^{E} \pm 2.33$
9	+	37% Phosphoric acid		$11.36^{E} \pm 1.48$

Different superscript letters indicate statistical differences ($p<0.05$)

Therefore, Dunnett's T3 test was performed. The controlled group (G1) demonstrated a significantly higher μTBS than the other groups that were contaminated by saliva.

Among the contaminated saliva groups (G2-G9), the group decontaminated by etching with 37% phosphoric acid (G4) provided the highest μTBS (31.02 ± 3.71 MPa). Drying (G2) or rinsing (G3) also presented high μTBS. However, the μTBS remained significantly lower than G4. The application of adhesive in G5-G9 also showed lesser μTBS than the groups that were decontaminated without using adhesive. The lowest μTBS was observed when the contaminated surface that was applied with Clearfil™ S3 Bond Universal (G7-G9).

Scanning electron microscopic showed similar resin composite surfaces between G1 and G2. A few craters were trapped in the resin composite surface in G3 and G4. The adhesive-applied groups (G5-G9) presented a mixture of adhesive (a) and resin composite (c) failures when the resin-resin interface was fractured by a universal testing machine (Fig.3).

Discussion

The result of this study showed that the controlled group, which was not contaminated by saliva, presented a significantly higher μTBS than the other groups. Saliva contamination caused a reduction in bond strength between the resin-resin interfaces. As demonstrated where every contaminated saliva group in this study presented significantly lower bond strength than the controlled group. When the saliva contacts the resin composite surface, the salivary glycoproteins can absorb into the surface, especially in the oxygen inhibited layer. So it can interfere with infiltration of the next resin composite layer to the contaminated layer.[10,11] Moreover, the saliva contained

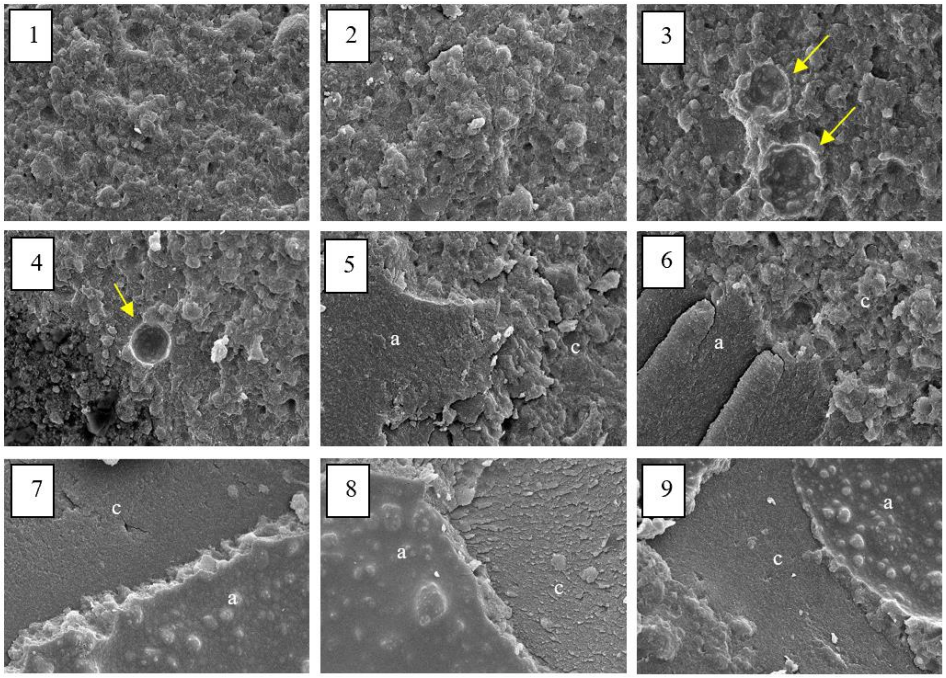

Figure 3. The scanning electron micrographs (2,000x) of fractured specimens of the different decontamination methods: numbers 1-9 correspond to groups G1 to G9 respectively. The arrows indicate the craters which were trapped in the resin composite surface; (a) - adhesive; (c) - resin composite.

99.4% water which also reduced the polymerization process of the resin composite.[12,13] Scanning electron micrographs of the fracture specimens supported the μTBS results. The rinsing group revealed a few craters that might have been caused by water, air, or saliva trapped in the saliva-decontaminated surface (Fig. 3-3).

Drying with air blow and rinsing with water showed similar values. However, both groups showed significantly lower bond strength than the etching with 37% phosphoric acid group. Although air-drying can evaporate the water in the saliva, the macromolecule can remain on the contaminated surface, wherein the pellicle of saliva can deposit into the resin composite in a short amount of contact time. Water-rinsing alone also cannot completely remove the salivary pellicle from the resin composite.

Etching on the saliva contaminated surface with 37% phosphoric acid has shown promising results. Ansari ZJ and Mohammadpour A also demonstrated that etching with 37% phosphoric acid can reestablish microshear bond strengths to nearly the control level.[14] Although the phosphoric acid cannot dissolve neither filler nor matrix of resin composite, and did not affect increased surface roughness, the phosphoric acid could denature the salivary protein and make it easy to remove by rinsing and drying. Furthermore, the phosphoric acid etching improved the wettability of the contaminated surface, providing a good attachment of the next resin composite layer to the resin composite layer.[15]

Many studies reported that the application of adhesive can improve the bond strength of repaired resin composite[16,17] because the adhesive can penetrate the microroughness of old resin composite which promotes surface area and allows increased contact between old and new resin composite.[18] However, this study provided different results. The application of both adhesive, Clearfil™ SE Bond or Clearfil™ S3 Bond Universal, showed significantly lower μTBS than the decontamination without adhesive-applied groups. Application of adhesive on top of contaminated resin composite might create a double oxygen inhibition layer which could have been produced by a sticky oxygen inhibited layer remaining on the contaminated surface and a oxygen inhibited layer that was polymerized on the newly adhesive-applied layer. This process could decrease the bond strength of resin-resin interfaces.

Based in this study, the application of adhesive might not be necessary for the decontamination method in which resin composite was immediately filled. Resin composite that has been filled for less than 1 month might still contain enough remaining unpolymerized monomers in the resin composite. This would allow existing resin composite to bond to the monomer in the next resin composite layer. The application of adhesive, especially the universal adhesive system containing water and alcohol, might increase contamination at adhesive applied areas.[19] In addition, the acidity of adhesive that might remain in the oxygen inhibited layer of the adhesive could contact the next layer of resin composite. This acidity could inhibit tertiary amine that acts as catalysts in the newly resin composite and interfere with the bond strength of resin-resin interfaces.[20]

In addition, the bond strength of the Clearfil™ S3 bond Universal applied groups demonstrated the lowest bond strength. Although the Clearfil™ SE Bond was affected by the acidity of MDP, this MDP was surrounded by a hydrophobic resin, which result in less impact on the next layer of resin composite.[21] So the adverse effect of acidity of Clearfil™ SE Bond might be lesser than Clearfil™ S3 Bond universal. This might result in adhesive-applied groups having lower bond strength than the untreated groups.

Conclusion

From the results of the experiments within the scope of this study, when saliva was contaminated between resin-resin interfaces, decontamination by rinsing and etching with 37% phosphoric acid for 15 seconds presented the highest mean microtensile bond strength and decontaminating by applying adhesive did not improve the bond strength.

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OM-00016

An *Oroxylum indicum* Extract, a Potential Biomaterial for the Treatment of Peri-implantitis

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ABSTRACT

Background and Objective: Flavonoids extracted from the *Oroxylum indicum* (OI) exhibit antibacterial, anti-inflammatory, antioxidant and antiadipogenic differentiation properties. The current study aimed to investigate effects of the extracts from the stem bark of the OI (OI/E) on an anti-inflammatory response and growth and osteogenic differentiation of the human periodontal ligament stem cells (hPDLSCs) to explore the applicability for the treatment of peri-implantitis.

Methods: The OI/E was initially refined, and the total flavonoid content and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assays were performed. After that the effects of the quercetin (QT) and luteolin (LT), the reference flavonoids and OI/E on growth and osteogenic differentiation of the hPDLSCs were investigated. Later, the hPDLSCs were stimulated with the 1 μg/ml bacterial lipopolysaccharide (LPS) and treated with OI/E, and then the levels of cell viability and secretion levels of the interleukin 1-β (IL-1β) and prostaglandin E2 (PGE_2) were measured.

Results: The initially refined OI/E contained significantly higher levels of flavonoid contents and DPPH scavenging activity than the crude extract. The in similar manners to QT and LT, the OI/E promoted growth and osteogenic differentiation of the hPDLSCs. Additionally, under the inflammatory stimulus from a bacterial toxin, the OI/E promoted cell growth, decreased levels of IL-1β secretion and promoted secretion levels of the PGE_2 of the LPS stimulated hPDLSCs (LPS-hPDLSCs).

Conclusion: The OI/E was a potential bioactive biomaterial that would decrease tissue destruction and promote bone regeneration of the peri-implantitis defects.

Keywords: Anti-inflammation, Human periodontal ligament stem cells, Natural flavonoids, *Oroxylum indicum*, Osteogenic differentiation

Introduction

A focus of the current study was on the *Oroxylum indicum* (L.) Kurz Extract, a Thai traditional medicinal plant that have antibacterial, antioxidative and anti-inflammatory effects and exhibit an inhibitory effect on adipogenic differentiation of the mesenchymal stem cells (MSCs),[1,2,3] in which an inhibitory effect on adipogenic differentiation could promote bipotent differentiation potential of MSCs toward osteogenic differentiation.[4] Dental implant has gained wide attention in restoring masticatory function and solve problems from tooth loss in elderly. Progressive bone resorption following tooth lost caused inadequate bone support for dental implant insertion. Together with limited availability of autologous bone and severity of bone deficiency, bioactive molecules are required to promote regenerative capacity of host bone and osteoconductive property of bone substitutes.[5] Quercetin(QT) and luteolin (LT) are the most abundant dietary flavonoids found in fruits and vegetables.[6] The QT and LT are flavonoids that appear to be the most potent among plant flavonoids in terms of their *in vitro* biological activities especially in promoting cell growth, anti-inflammatory and anti-oxidative activity and stimulate osteoblastic differentiation.[7,8] Consequently, the current study used quercetin as a positive control.

Additionally, functions and survival of dental implant are threatened by peri-implantitis, a complication jeopardizing dental implant survival. Causes of peri-implantitis associate with bacterial toxins and inflammation of the supporting gingiva, periodontal ligament and bone around the implants.[9] A treatment protocol of peri-implantitis includes mechanical debridement with or without systemic or local antibiotic treatment, and a need for additional regenerative bone surgery are determined upon the extent of bone resorption.[10] Based on anti-inflammatory, antioxidant and anti-adipogenic differentiation properties of the extract from the stem bark of the OI (OI/E), the OI/E might be able to decrease tissue damages caused by bacterial toxin and inflammatory cytokines and promote bone regeneration in the peri-implantitis defects. It was hypothesized that the OI/E would accelerate healing of the tissues and bone regeneration surrounding the dental implants. Therefore, the current study aimed to investigate effects of the OI/E on an inflammatory response and growth and osteogenic differentiation of the human periodontal ligament stem cells (hPDLSCs) to explore the applicability of the OI/E for the treatment of peri-implantitis.

Materials and Methods

Preparation of the Extracts from Stem Bark of the OI (OI/E)

Initially refined OI/E were a gift from Assoc. Prof. Dr. Supakorn Boonyuen, the Department of Chemistry, Faculty of Science and Technology, Thammasat University, Thailand. In brief, barks of the OI from Buriram province in a Northeastern region of Thailand were peeled, minced, washed in deionized (DI) water, air dried, and crushed into powder. Then, about, 10 g of the cleaned powder was dissolved in 100 mL of DI water and boiled for about 20 min. After that, the solution was filtered and stored at 4 °C as a crude extract and kept at -20ºC for further use. Later, the crude extracts were initially refined using the chromatography technique.[11,12]

Characterization of the Extracts from the Stem Bark of the OI (OI/E)

Total Flavonoid Content (TFC) Assay

A total flavonoid content (TFC) assay uses an aluminium chloride colorimetric method to estimate levels of flavonoids in the extracts. In brief, the samples, OI/E and crude (Cr) in DI water were mixed with the 5% (w/v) sodium nitrite, followed by the 10% (w/v) aluminum chloride and 4%(w/v) sodium hydroxide, respectively (All chemicals from Sigma-Aldrich, St Louise, MO). Then, the solutions were changed to brown and dark brown colors and the absorbance was read at 510 nm using a microplate reader (Varioskan Flash, Thermo Fisher scientific, Vantaa, Finland). After that the total flavonoid content of samples were extrapolated from a standard curve of QT and expressed as equivalent to quercetin concentration in ppm.[13,14]

DPPH Radical Scavenging Assay

The free radical activity was determined by measuring the ability of the extracts to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The principle is based on the reduction of the DPPH in the presence of a hydrogen donating antioxidant that the reduction reactions decrease color of the DPPH in relation to the proton donating ability or antioxidant capacity of the samples.[3,15] The samples were mixed with the DPPH solution (Sigma-Aldrich) and allowed to stand for 30 minutes in the dark at room temperature (RT). Then the absorbance was measured at 515 nm wavelength using a microplate reader (Varioskan Flash), and methanol was use as a blank solution. The radical scavenging activity was expressed as the inhibition percentage using the equation, where A_0 and A_1 were absorbances of the control and sample solutions, respectively, as follows.

DPPH scavenging activity (%) = $[(A_0 - A_1) / A_0] \times 100$.

Where A_0 was the Absorbance of control reaction and A_1 was the Absorbance in presence of test or standard sample.[15]

Human Periodontal Ligament Stem Isolation and Expansion for the Investigations

This study was approved by the human subject ethics board of Thammasat University, the Ethical Review Sub-Committee Board for Human Research Involving Sciences, No.3 (Protocol number 102/2563) and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013 and under patient informed consent. A total of 12 wisdom teeth were collected from patients undergoing extraction or surgical removal of the whole teeth at the Dental Clinic, Thammasat University Hospital. Patients were 18 – 25 years-old and healthy without any systemic diseases or not currently taking any medications.

Then in brief the periodontal tissue was scraped from the 2/3 of roots, minced, washed in phosphate buffer saline (PBS) and incubated in 3 mg/ml collagenase and 4 mg/ml dispase (Sigma-Aldrich) and then the dissociated cells and tissues were cultured on the cell culture flasks, 2 teeth per 25 cm^2 flask. After that the expanded cells at passage 1 from 3 patients were mixed as one batch for using in the further investigations. Growth medium comprised of DMEM-F12 with phenol red, 10% Fetal bovine serum (FBS), 1% penicillin/streptomycin and 0.5% fungizone (all from Gibco, Thermo Fisher Scientific, Waltham, MA). Culture medium was changed every 2 days.[16]

Cell Culture for the Investigations

Human PDLSCs were seeded at a density of $3x10^3$ cells/cm^2 for the investigations on cell growth and anti-inflammatory assays and $5x10^3$ cells/cm^2 for osteogenic differentiation in a monolayer cell culture. Osteogenic medium comprised of 10 mM β-glycerophosphate, 50 mM L-ascorbic acid 2-phosphate, and 100 nM dexamethasone (all from Sigma-Aldrich).[17] For experiments on anti-inflammatory responses, after 24 h incubation a regular growth medium was changed to growth medium with 1% fetal bovine serum (FBS) supplemented with QT, LT, OI/E according to groups of the study. For a study on osteogenic differentiation, the hPDLSCs were cultured in osteogenic (OS) medium with 10% FBS.

Cell Viability Assay

Cell viability assay was performed to determine cell cytotoxicity and working concentrations of the QT, LT, and OI/E. The hPDLSCs were incubated with the 1.25 – 10 ppm QT, LT and 1.25-20 ppm OI/E in growth medium for 72 h. Then, cell viability assay was performed using CellTiter 96® AQueous One Solution (Promega, Madison, WI) following the manufacturer's instruction. Absorbances were measured at 490 nm using microplate reader (Varioskan Flash).[18] Then, the selected concentrations were used as working concentrations for the following investigations.

Secretion of an Inflammatory Cytokine, Interleukin-1β and Prostaglandine E_2

To activate the inflammatory response, hPDLSCs were cultured with the 1 µg/ml purified *Porphyromonas gingivalis* lipopolysaccharide (LPS) (InvivoGen, San Diego, CA) (LPS-hPDLSCs) in growth mediums supplemented with and without OI/E, and QT for 72 h.[17] Then, levels of interleukin-1β (IL-1β) and prostaglandin E2 (PGE_2) were measured using the Quantikine ELISA kit (R&D systems, Minneapolis, MN) and PGE_2 ELISA kit (Enzo, USA), respectively following the manufacturer's instructions and the absorbances were measured at 450 and 405 nm respectively using a microplate reader (Varioskan Flash).[18,19]

Osteogenic Differentiation

Measuring Levels of Alkaline Phosphatase Activity and Calcium Contents

At each investigation time hPDLSCs, on cell culture plates were lysed in an ice cold 1% Triton X-100 (Sigma-Aldrich). The total protein contents in cell lysis solutions were measured using DC protein assay kit (Bio-rad, Hercules, CA). Levels of alkaline phosphatase (ALP) activity in cell lysate solutions were measured using the ALP yellow liquid substrate system for ELISA (Sigma-Aldrich) following the manufacturer's instructions.

For measuring levels of calcium contents in the extracellular matrix (ECM), cell pellets collected from the cell lysis procedure were washed two times in PBS and incubated in 0.5 M hydrochloric acid in PBS (Sigma-Aldrich) at 37°C for 24 h. Then levels of calcium contents were measured using a calcium colorimetric assay kit (Sigma-Aldrich) following a manufacturer's protocol. After that levels of ALP activity and calcium contents were reported as nanomolar p nitrophenol and nanogram calcium per milligram total protein contents, respectively.[20]

Statistical Analysis

Numbers of samples at each investigation time were 3-5 samples and reported as Mean ±. SD. Data were analyzed using SPSS

Statistics for Windows, Version 26.0 (IBM Corp, Armonk, NY). Kolmogorov-Smirnov test was used to examine the data distribution. One-way ANOVA and Scheffe's test (Tukey's HSD) were used for normal distribution data. For non-normality distribution Kruskal–Wallis and Dunn-Bonferroni post hoc tests were used for multiple comparisons. Significant difference was set at *p*-value < 0.05 (N=5, Mean ± SD).

Results

Effect of the Reference Flavonoids on Cell Growth and Osteogenic Differentiation of human periodontal ligament stem cells

Effects of the Reference Flavonoids and Extracts on Cell Growth

It was found that when the hPDLSCs were cultured in growth medium supplemented with the 0 -10 ppm QT and LT, levels of cell viability of the hPDLSCs with 5 ppm QT was significantly higher than a control in a regular growth medium (Ctr) (p<0.05) and LT exhibited an adverse dose dependent effect on cell viability. The levels of cell viability in the 10, 5 and 2.5 ppm LT were significantly lower than the 1.25 ppm LT and control groups, and the levels of cell viability were significantly decreased with the increase of concentrations of LT (p<0.05). It should be noted that the levels of cell viability in 10-0 QT groups were stable and not significantly different, and a decrease of cell viability in the 5 ppm LT group was lower than 55% of the control (54.82± 4.16) (Fig. 1A).

Effects of the Reference Flavonoids on Osteogenic Differentiation

Then, the effects of the 2.5 and 5 ppm QT and LT on levels of ALP activity were investigated. It was found that levels of the ALP activity of the 2.5 ppm QT and LT were significantly higher than the control and 5 ppm groups, and the levels in QT and LT groups at each concentration were not significantly different (Fig. 1B).

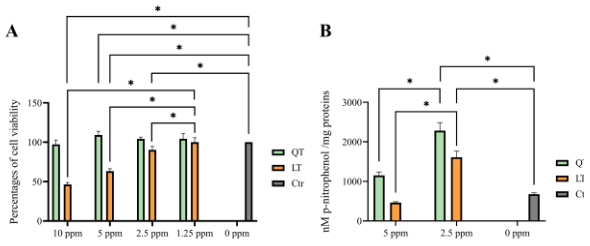

Figure 1. Demonstrating effects of quercetin (QT) and luteolin (LT) on cell growth and osteogenic of the human periodontal ligament stem cells (hPDLSCs). Human PDLSCs were cultured in growth medium supplemented with the QT and LT for 72 h. (A) Percentages of cell viability and (B) Levels of alkaline phosphatase (ALP) activity. The percentages of cell viability were calculated relatively to the distinct optical density level (OD) of a control group in growth medium alone (Ctr). A symbol * represents significantly different between groups (N=5, Mean ±SD).

Characteristics of the *Oroxylum Indicum* Extracts

The flavonoid content (TFC) and the DPPH scavenging assays were performed to estimate levels of the flavonoid contents and antioxidant activity of crude extract and the initially refined extracts from the second (F2), third (F3) and fourth (F4) chromatography fractions (F). It was found that the average flavonoid contents of the initially refined extracts (F2, F3 and F4) was equivalent to 3.45±0.09 ppm and the crude, 3.36±0.12 ppm of QT, respectively. The TFC levels of the F3 at 3.84±0.16 ppm of QT was significantly higher than the F4 and crude (p<0.05) (Fig. 2A).

The crude and extracts Fraction 2, 3 and 4 exhibited high levels of antioxidant property at 78.46±0.59 % DPPH, in which the levels of the activity of the initially refined extracts, F2, F3 and F4 were significantly higher than a crude group (p<0.05) (Fig. 2B).

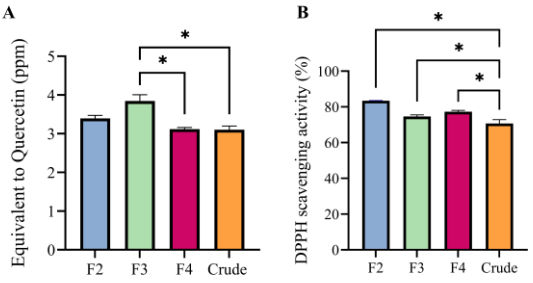

Figure 2. Demonstrating levels of total flavonoid contents and antioxidant capacity of the initially refined and crude extracts from the *Oroxylum Indicum* (OI), (A) Total flavonoid content assay (TFC) and (B) DPPH Radical Scavenging Assay. Abbreviations F2, F3 and F4 denote the initially refined extracts (OI/E) from the second, third and fourth fractions of the chromatography technique and Crude, the non-refined extract. A symbol * represents significantly different between groups (N=5, Mean ± SD).

Working Concentrations of the Initially Refined *Oroxylum Indicum* Extracts

The 0 – 20 ppm OI/E and 1.25 – 5 ppm QT were noncytotoxic to the hPDLSCs. The percentages of cell viability of the 0 – 20 ppm OI/E (104±10.59%) and 1.25 – 5 ppm QT groups (110±4.77%) were not significantly different from the control in a growth medium alone (p>0.05). The 10 and 20 ppm QT exhibited an inhibitory effect on cell viability that the percentages of cell viability of those groups were lower than 40% of the control (p<0.05) (Fig. 3).

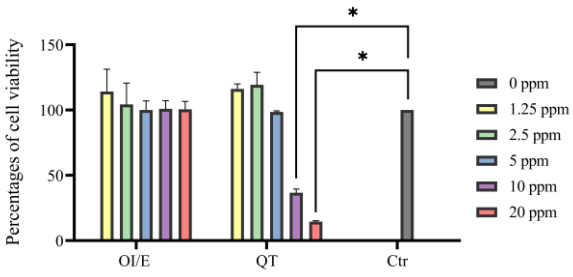

Figure 3. Demonstrating effects of the initially refined *Oroxylum Indicum* extracts (OI/E) and quercetin (QT) on cell growth of the human periodontal ligament stem cells (hPDLSCs). The hPDLSCs were cultured in growth medium supplemented with the OI/E and QT in growth medium for 72 h. The percentages of cell viability were calculated relatively to the distinct optical density level (OD) of a control group in growth medium alone (Ctr). A symbol * represents significantly different between groups (Ctr) (N=5, Mean ±SD).

Effect of the Initially Refined *Oroxylum Indicum* Extracts on the Osteogenic Differentiation of the Human Periodontal Ligament Stem Cells

The OI/E promoted osteogenic differentiation of the hPDLSCs in a dose dependent manner. When the hPDLSCs were cultured in osteogenic mediums supplemented with the OI/E and QT for 14 days, it was found that levels of the ALP activity and calcium contents of the 20 ppm OI/E group were significantly higher than a control group (p<0.05), while the levels of the 2.5 and 5 ppm QT tended to be higher than control and lower than the 20 ppm OI/E group (p>0.05) (Fig. 4A and B).

Effect of the Initially Refined *Oroxylum Indicum* Extracts on Secretion Levels of the Interleukin-1β and Prostaglandin E2

The hPDLSCs were stimulated with the 1 ug/ml LPS for activate the inflammatory response, (LPS-hPDLSCs) then levels of the IL-1β and PGE_2 in a culture medium were measured. The LPS tended to

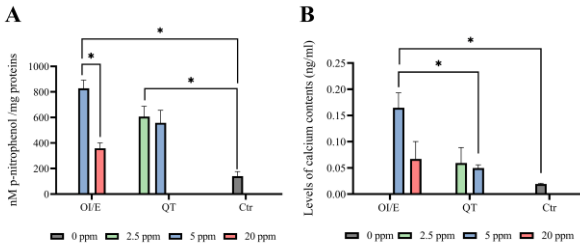

Figure 4. Demonstrating promoting effects of the initially refined *Oroxylum Indicum* extracts (OI/E) and quercetin (QT) on osteogenic differentiation potential of the human periodontal ligament stem cells (hPDLSCs), (A) Levels of alkaline phosphatase (ALP) activity and (B) Levels of calcium contents. The hPDLSCs were cultured in the osteogenic medium supplemented with the OI/E and QT for 14 days. Abbreviation, Ctr denotes a control group in growth medium alone. Symbols * represents significantly different from the other group (p<0.05) (N=5, Mean ± SD).

increased levels of IL-1β in all groups, control, QT and OI/E (p>0.05), and the levels of 20 ppm OI/E with and without LPS were markedly and significantly lower than a control group (p<0.05). The levels of the QT groups with and without LPS were lower than control but higher than the OI/E groups, but they were not significantly different (p>0.05) (Fig. 5A).

Regarding levels of PGE_2, in a control group, the LPS stimulation significantly decreased levels of PGE_2 (p<0.05). In contrast, in the OI/E and QT groups, the LPS stimulation significantly increased levels of the PGE_2 (p<0.05) and that the levels of the LPS-hPDLSCs in growth medium were significantly lower than the OI/E and QT groups (p<0.05) (Fig. 5B).

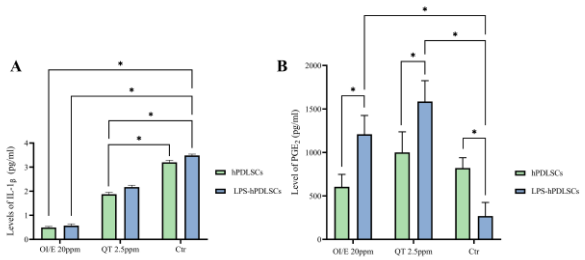

Figure 5. Demonstrating effects of the initially refined *Oroxylum Indicum* extracts and quercetin (QT) on secretion levels of an inflammatory cytokines, interleukin 1β (IL-1β) and prostaglandin E2 (PGE_2) of the human periodontal ligament stem cells (hPDLSCs), (A) Levels of IL-1β and (B) PGE_2. The hPDLSCs were cultured in growth medium without (hPDLSCs) and with the 1 µg/ml bacterial lipopolysaccharide stimulated (LPS) (LPS-hPDLSCs) for 48 h. A symbol * represents significantly higher than the other group (p<0.05) (N=5, Mean ± SD).

Discussion

The current study exhibited the non-cytotoxicity and dose dependent effects of the OI/E on promoting osteogenic differentiation of the hPDLSCs that corresponded with the reference flavonoids, QT and LT. For the better results, the findings suggested that the initially refined extract should be used, as it was shown that the levels of flavonoid contents and antioxidative property of the initially refined extracts were higher than those of crude, and the sequence of fractions could affect therapeutic efficacy of the extracts.

Anti-oxidative stress and anti-inflammatory properties of the OI/E has been reported[3,20] but those effects on hPDLSCs including the effects on growth and osteogenic differentiation of the hPDLSCs have not been investigated. The findings agreed with the previous reports that the natural flavonoids, such as QT and LT could promote growth and osteoblastic differentiation of the mesenchymal stem cells[6,8,21] and highlighted a strong promoting effect of the OI/E on hPDLSCs. As it could be seen that the promoting effects of the 5 ppm OI/E on osteoblastic differentiation of hPDLSCs were stronger than the 5 ppm QT, including the decreasing of an inflammatory cytokine, IL-1β.

Furthermore, other new findings from the current study were that the stem bark extract from the *Oroxylum indicum* (OI/E) exhibited antioxidative activity and anti-inflammatory effect on the LPS-hPDLSCs as shown by the percentages of DPPH scavenging activity and the decreasing of levels of IL-1β, an inflammatory cytokine, respectively. Additionally, the OI/E could promote growth and osteogenic differentiation of the hPDLSCs, as demonstrated by the increasing of early and late osteoblastic differentiation markers, ALP activity and levels of calcium contents, respectively.[22] Furthermore, the biologic effects of the OI/E tended to be higher than those of QT, a well-known and abundant flavonoid in fruits and vegetables.[8,23] Quercetin is a natural antioxidant found in various fruits and tea leaves[6] that can minimize a cellular damage caused by free radicals, and promote cell growth and osteoblastic differentiation of the mesenchymal stem cells (MSCs).[21]

It was interesting that when the hPDLSCs were stimulated by the LPS (LPS-hPDLSCs) in the presence of the OI/E and QT, the PGE_2 level was increased, while it was decreased in a regular growth medium group. Chen and co-workers in 2019 demonstrated and explained that prostaglandin E_2 (PGE_2) is an important inflammatory cytokine secreted following cell membrane injuries. It can stimulate the inflammatory cascades and promote vasodilation and also osteoblastic differentiation by activating cAMP signaling through G protein-coupled EP4 receptor.[22] Therefore, it could be postulated that during an inflammation stimulated by bacterial toxin in periodontitis and peri-implantitis the OI/E would be able to promote osteoblastic differentiation of the mesenchymal stem cells and enhance bone regeneration.

As peri-implantitis is an inflammatory disease attributed mainly by bacterial toxins that stimulate excessive inflammatory cytokine causing damages to peri-implant tissues leading to attachment loss and bone resorption,[23] the synergistic effects of an antibacterial property of the OI/E[24] and together with the protective and regenerative properties of the OI/E found in the current study, the OI/E would be able to decrease damages to cellodontium and promote bone regeneration in the peri-implantitis defects.

Conclusion

The initially refined *Oroxylum indicum* crude extracts (OI/E) supported cell growth, decreased secretion of an inflammatory cytokine, and promote osteogenic differentiations of hPDLSCs in a dose dependent manner. The initially refined *Oroxylum Indicum* extracts was a potential bioactive biomaterial that would decrease tissue destruction and promote bone regeneration of the peri-implantitis defects.

Acknowledgements

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Comparison of Fracture Resistance and Failure Mode of Self-adhesive Composites: Pilot Study

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ABSTRACT

Background and Objective: Renewal MI (RMI) is a new self-adhesive composite resin developed from the users' perspectives as alternative filling material for special needs patients. This study aimed to examine fracture resistance of Renewal MI in comparison to the conventional composite resin, conventional glass ionomer, and Renewal MI with adhesive, when subjected to thermocycling at 5,000 and 10,000 cycles.

Methods: Fifty premolars were divided into ten groups (N=5/group). The control group (CT) was intact teeth and negative control group were filled with IRM. Except for CT group, the class II MOD cavity was prepared and underwent chemically-induced caries process. Groups 1 was restored with Filtek Z350XT; groups 2 – Fuji IX; groups 3 - RMI and groups 4 - RMI and Universal adhesive. Five teeth/group were randomly assigned to thermocycling process at 5,000 cycles and another five teeth/group at 10,000 cycles. Specimens were tested under the Universal testing machine using a rounded stainless-steel diameter-3-mm tip. One-way ANOVA and Bonferroni testes were used to determine difference between groups. Independent T-test was used to determine difference between the thermocycling 5,000 and 10,000 cycles.

Results: There was no statistical difference between four experimental groups, either at 5,000 or 10,000 cycles. Comparison within material group, between at 5,000 and 10,000 cycles, also showed no significant difference. The least number of unfavorable failure was found in composite resin groups.

Conclusion: Renewal MI, with or without adhesive, restored in MOD cavities and demineralized dentin, has comparable fracture resistance to conventional restorative materials, but has higher ratio of unfavorable failure.

Keywords: Class II MOD, Demineralized dentin, Fracture resistance, Thermocycling, Self-adhesive composite

Introduction

To overcome difficulties in restoring caries for special health care need (SHCN) patients, high performance restorative materials with simple handling technic must be selected to suit the limitations dentists often encountered. Complete caries removal, ideal cavity preparation and incremental filling might be unable to achieve, without general anesthesia setting.[1] Restorative material used in SHCN patients should be optimized strength, less technical sensitive, controllable setting and able to bond to demineralized dentin. Restorative procedure should not provoke anxiety.[1] The atraumatic restorative treatment (ART) approach has been demonstrated to be acceptable, feasible and effective technic for restoration in this population.[1]

Various composite resins have been the popular choices, due to their better mechanical properties, compared to other direct restorative materials. However, disadvantages of composite resin were listed, such as polymerization shrinkage and being hydrophobic.[2] Clinically, failure features observed after composite resin restorations were mainly secondary caries and fracture of tooth/material.[3,4] High Viscosity Glass Ionomer (HVGI) material is developed with improved mechanical properties while remaining biocompatible and bioactive.[5] HVGI is becoming a choice for restoration, in SHCN patients that counterbalances the undesirable properties of composite resin.[6]

Recently, injectable composite resin has been designed, based on the perspective of a group of child patients and pediatric dentists at the UCL Eastman Dental Institute, UK. Renewal MI (RMI) was developed to be an alternative material that was bioactive, antibacterial, self-adhesive and easy to use. Monomer modification resulted in reduced monomer conversion. Properties, such as wear resistance, biaxial flexural strength (BFS) and modulus (BFM) was demonstrated to be comparable to the commercially available composite resins.[7] When submersed in artificial saliva, RMI could expand and seal the tooth-material interfaces, compensating the polymerize shrinkage. Additionally, silicate glass in combination with hybrid fillers including, monocalcium-phosphate and polylysine, not only increased the strength, but also were bioactive that could induce precipitation on the material's surface and in the crack within material bulk, and releasing mild antibacterial effect.[7] Further studies were conducted in extracted teeth to demonstrate that calcium-phosphate precipitation gradually sealed the tooth-material interface resulted in reduced microleakage after the restoration aged in the artificial saliva.[8]

Post-operative durability of teeth restored with RMI has not been examined. The aging procedure is generally recommended prior to mechanical testing of any biomaterials. Different coefficients of thermal expansion between tooth structure and the filling material, produce by thermocycling process, give the effect

mimicking the usage of restoration in oral cavity.[9] This study aimed to evaluate the fracture resistance of RMI restoration after being subjected to two thermocycling conditions, in comparison to conventional composite resin (Filtek Z350XT) and HVGI (Fuji IX capsule). It could also be hypothesized that self-etched Universal adhesive may strengthen the RMI restoration and improve the outcome in a longer thermocycling test.

Materials and Methods

Sample

Fifty maxillary premolars were collected. The teeth were cleaned, then stored in 0.1% Thymol to be used within 6 months. This study was approved by the Khon Kaen University Ethics Committee for Human Research, Thailand (Protocol Number HE652067).

2.1 Cavity preparation

Mesio-occluso-distal (MOD) cavities were prepared using a round inverted cone bur (364R, Shofu) with high speed and water spray. For every 5 teeth, new bur was changed. One researcher (JR) prepared all samples, which were checked under magnification glass by another co-worker (PP).

Dimensions of the cavity was as follows, occulsal isthmus width was 1/3 of intercuspal distance; lingual wall depth 2 mm, axial wall depth 1.5 mm, gingival floor width 1 mm. All internal line angles were rounded.

2.2 Artificial caries formation; Initial demineralization

Each specimen was covered with nail varnish except for 1 mm. surrounding margin of the cavity. Root apexes were blocked using sticky wax. Teeth were immersed in demineralization solution at the volume of 10 ml/tooth at 37°C for 3 days. The pH of solution was maintained at 5, by monitoring daily with pH meter. The demineralization solution composed of $CaCl_2$ 166.485 mg/L, KH_2PO_4 122.477 mg/L, NaN_3 325.05 mg/L, Acetic acid 2.865 ml/L in 1,000 mL of deionized water. NaOH was used to adjust pH level to 5.[10]

2.3 Restorations

The teeth were randomly divided into 5 teeth/group and rinse thoroughly with distilled water before restoration. Tofflemire band was placed in a Tofflemire matrix retainer.

2.3.1 Filtek Z350 XT + Single Bond Universal Adhesive group (Group 1a and 1b)

Adhesive applied to air-dried surface with agitating action for 20 seconds following by medium air pressure applied for 5 seconds and cured for 10 seconds. (LED.E light curing, Guilin Woodpecker Medical Instrument Co.,LTD, Chaina) The procedure was repeated to create double-layer application. Composite resin shade A2 (Filtek Z350 XT; 3M ESPE) was placed using incremental technic. Thickness of each layer was 1.5-2 mm. and cured for 40 seconds and another 40 seconds after matrix band removal.

2.3.2 Fuji IX GP Extra (Group 2a and 2b)

Dentine conditioner applied on for 10 seconds and rinsed thoroughly. The cavity was dried without desiccating by gently blowing. Capsule was activated and mixing using amalgamator for 10 seconds (4,000RPM) before loaded onto the GC Capsule Applier. Glass ionomer was injected into the cavity. When glass ionomer was set approximately 6 minutes after start of mixing, the restoration was polished and coated with petroleum jelly.

2.3.3 Renewal MI (Group 3a and 3b) composition showed in Table 1.

After gently air blowing, Renewal MI was applied into the cavity using dispenser and cured for 40 seconds and another 40 seconds after removal of matrix band.

Table 1. Shows the main composition of Renewal MI (Schottlander, Letchworth, UK) used in this study.

Component	Function
urethane dimethacrylate (UDMA)	Base monomer (18 wt%)
polypropylene glycol dimethacrylate (PPGDMA)	Diluent monomer (6 wt%)
4-methacryloxyethyl trimellitic anhydride (4-META)	Adhesive monomer (0.75 wt%)
silane treated aluminosilicate glasses	Radiopaque filler (66 wt%)
mono-calcium phosphate (MCP)	Remineralising agent (6 wt%)
ε-poly-L-lysine (PLS)	Adhesion promoter (3 wt%)
camphorquinone (CQ)	Initiator (0.25 wt%)

2.3.4 Renewal MI + Single Bond Universal Adhesive (Group 4a and 4b)

Double-layer adhesive was applied by the similar procedure in 2.3.1 and the cavity was filled with Renewal MI using similar procedure in 2.3.3.

Five sound teeth were assigned in the control group, and five negative control teeth were restored with IRM.

Samples were stored in artificial saliva (composition in 2.4) at 37°C for 24 hours, then finishing and polishing with white stone and Enhance finishing bur. Another blinded researcher (PP) checked for quality of the restoration. Defective samples were excluded.

2.4 Maturation and ageing process

Varnish and sticky wax were gently removed by acetone solution, prior to maturation process. The artificial saliva composed of KCL 0.625 g/L, $MgCl_2 \bullet 6H_2O$ 0.059 g/L, $CaCl_2$ 0.166 g/L, KH_2PO_4 0.326 g/L, K_2HPO_4 0.804 g/L, Sodium benzoate 2 g/L and Sodium Carboxy methyl Cellulose 10g/L in 1000 mL of deionized water. 1 molar solution of Hydrochloric acid (HCl) was used to adjust pH level to 7.[11] All samples were immersed in artificial saliva at the volume of 10 mL/tooth at 37°C for 28 days. Newly prepared artificial saliva was replaced every week.

Samples were subjected to thermocycling at 5°C and 55°C with 30 seconds dwell time and 10 seconds for transfer for group (a) 5,000 cycles and group (b) 10,000 cycles.

2.5 Blinding

Samples were mounted into acrylic resin blocks. The cementoenamel junction (CEJ) was 1 mm. above the surface of acrylic resin. Samples were labelled (1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b) and covered before randomly re-distributed for the order of testing by a researcher (PP).

2.6 Fracture resistance test

For a static load test, sample was placed firmly in a stabilizing ring. Under Universal testing machine (Lloyd LRX, Lloyd Instruments Ltd, UK), vertical compressive force was delivered using a rounded stainless-steel testing rod (diameter 3 mm.), with a crosshead speed at 1 mm/min. The compressive force, measured in Newtons (N), was applied onto the central fossae along the tooth long-axis, linearly until fracture automatically detected.

2.7 Failure Modes

Under a stereomicroscope (Stereo-microscope, Nikon, measure scope 20), fractured specimens were evaluated and calibrated by 2 operators (JR and PP). Characteristics of fracture were divided into two modes; 1) favorable; superficial fracture (delamination) of restoration, cohesive fracture of the tooth structure, such as damage chip or crack or fragment, but only those with fracture line ended above the CEJ. 2) unfavorable (Catastrophic); cohesive fracture of the tooth structure with the fracture line extending towards the root and fractures of the tooth and/or restoration with the fracture line ending below the CEJ[12,13] (Fig. 1).

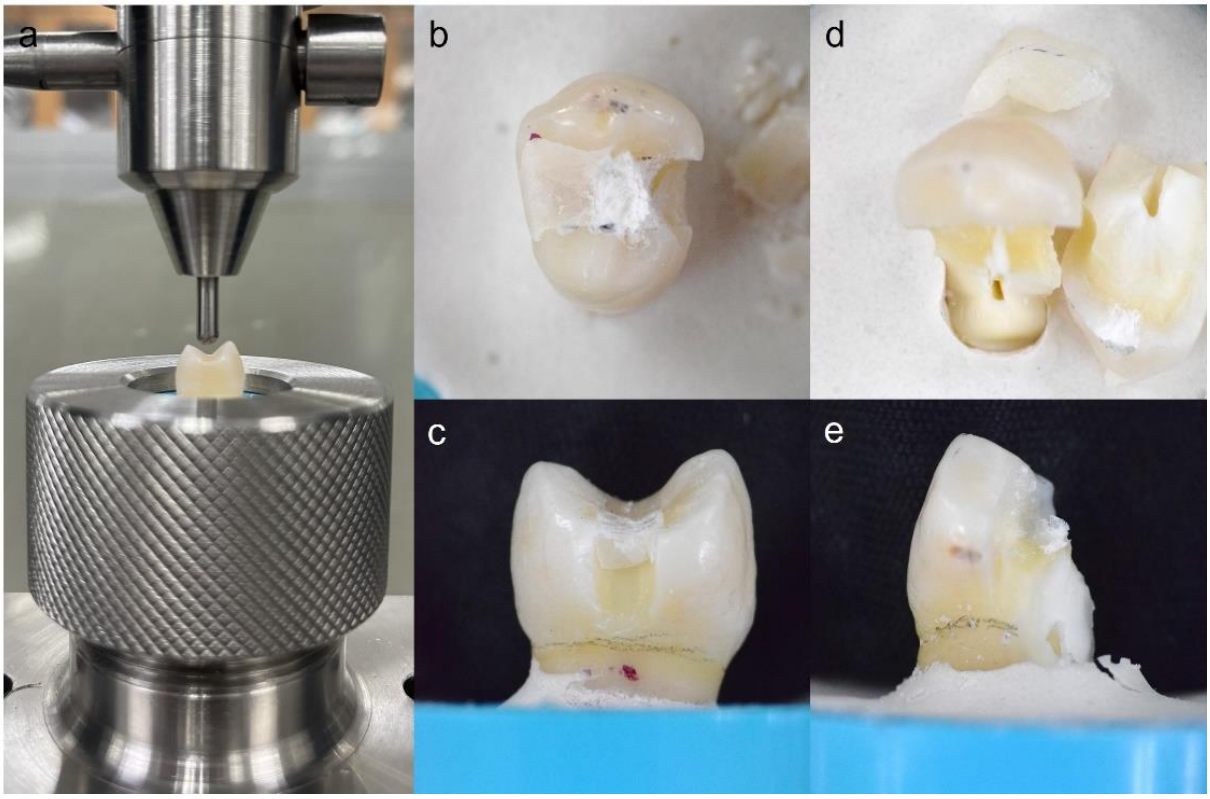

Figure 1. Shows examples of failure modes from Fracture resistance loading. a) the specimen tested using a rounded stainless-steel testing rod diameter 3 mm. b, c) **Favorable Failure** on occlusal and side views, show fracture line above the CEJ. d, e) **Unfavorable Failure** (Catastrophic) on occlusal and side views, show the fracture line beneath the CEJ.

2.8 Statistic analysis

Data analysis performed using SPSS version 21 (Ver. 21.0, SPSS, Chicago, USA). Data were showed as mean and standard deviation (SD), median and frequency (%). Shapiro-Wilk test was used for testing the normality of continuous variables. Data were analyzed with one-way ANOVA followed by Post-hoc test (Bonferroni). The comparison of each restorative material between 5,000 and 10,000 cycles was tested using the independent t-test. The statistic significant level was established at p-value < 0.05.

Results

The mean of fracture resistance of the all groups, at thermocycling 5,000 and 10,000 cycles are presented in Table 2. The highest mean fracture resistance was noted in sound teeth in the control group (CT group) at 1513.8 N and the lowest was in the IRM group at 757.8 N. In groups subjected to thermocycling at 5,000 cycles, teeth restored with Renewal MI and adhesive (RMI+U) had the highest fracture resistance force, followed by conventional Renewal MI (RMI), glass ionomer (GI) and composite resin (Com+U). After 10,000 cycles, the highest mean fracture resistance was found in RMI group, followed RMI+U, Com+U and GI groups.

At 5,000 cycles there was no statistical significance found among the 4 experimental groups. The analysis further showed that significant difference was observed when RMI+U, RMI, or GI group was compared to the IRM group (p-value<0.05). Although fracture resistance force of the Com+U group was higher than those in IRM group, no statistical significance was noted (p-value=0.214) (Table 3). At 10,000 cycles, the difference between fracture resistance force among 4 tested materials was not statistically significant. However, when compare to the IRM group, the force of either the Com+U, RMI+U or RMI group was significantly higher. The force of GI group was higher than the IRM group, but there was no statistical significance (Table 4).

Fig. 2. shows distribution of unfavorable failure samples, comparing each material between 5,000 and 10,000 cycles. All samples restored with IRM were fractured in unfavorable modes. Three out of 5 of the control teeth were also unfavorable fractured. Fracture lines of all tested groups were defined as mix type, where segment lines involved the tooth structure and materials. The unfavorable samples of Renewal MI was increased from 2 samples,

Table 2. Fracture resistance force (Newton) of different restorative materials at 5,000 and 10,000 thermo-cycles, in mean value, standard deviation (S.D.), maximum, minimum and median.

Group (N=5)	Mean ± SD	Mean ± SD	Median	
	5,000C	10,000C	5,000C	10,000C
Com+U	1129.6 ± 245.23	1367.4 ± 332.50	1253	1328
(Max-Min)	(1371-864)	(1783-951)		
GI	1278.4 ± 223.24	1185.8 ± 202.79	1287	1244
(Max-Min)	(1546-991)	(1364-840)		
RMI	1314.2 ± 121.10	1407.4 ± 149.64	1280	1493
(Max-Min)	(1443-1191)	(1521-1185)		
RMI+U	1411.4 ± 231.02	1386.4 ± 227.23	1476	1488
(Max-Min)	(1626-1103)	(1555-1009)		
CT	1513.8 ± 292.57		1553	
(Max-Min)	(1822-1031)			
IRM	757.8 ± 182.87		784	
(Max-Min)	(956-472)			

Groups: Com+U = Z350 and Universal adhesive, GI = Fuji IX GP Extra, RMI = Renewal MI, RMI+U = Renewal MI and Universal adhesive, CT = normal teeth, IRM = IRM

Tables 3. Significance of the mean of fracture resistance between groups at 5,000 thermo-cycles.

Group	Com+U	GI	RMI	RMI+U	CT	IRM
Com+U	-	p=1.000	p=1.000	p=0.850	p=0.175	p=0.214
GI	-	-	p=1.000	p=1.000	p=1.000	p=0.017*
RMI	-	-	-	p=1.000	p=1.000	p=0.009*
RMI+U	-	-	-	-	p=1.000	p=0.002*
CT	-	-	-	-	-	p<0.001*

* mean difference significant at p-value ≤ 0.05 level.; Bonferroni test

Tables 4. Significance of the mean of fracture resistance between groups at 10,000 thermo-cycles.

Group	Com+U	GI	RMI	RMI+U	CT	IRM
Com+U	-	p=1.000	p=1.000	p=1.000	p=1.000	p=0.007*
GI	-	-	p=1.000	p=1.000	p=0.610	p=0.141
RMI	-	-	-	p=1.000	p=1.000	p=0.004*
RMI+U	-	-	-	-	p=1.000	p=0.005*
CT	-	-	-	-	-	p=0.001*

* mean difference significant at p-value ≤ 0.05 level.; Bonferroni test

at 5,000 cycles to become 5 samples after 10,000 cycles. When adhesive used, the unfavorable of RMI+U groups were observed in 4 teeth at both 5,000 and 10,000 cycles. Similarly, the number of unfavorable samples of the GI groups were 3 teeth at both 5,000 and 10,000 cycles. In the Com+U groups, the unfavorable failure samples were limited to 1 and 2 teeth at 5,000 cycles and 10,000 cycles, respectively.

Discussion

When functioned in oral cavity, performance of any restorations would be challenged by a critical force from mastication. The force simultaneously effects on the tooth structure, interface layer and restorative material, as a whole. Fracture resistance has been a practical laboratory model to be primarily implemented, for predicting the longevity of restorations.[14] High intensity static loading, usually higher than mastication force, is exerted through the occlusal to determine the reaction of the restored tooth to such force.[15] The reactive stress distributed through buccal and lingual cusps, on the occlusal central fissure, as well as on the cervical segment of the enamel and dentin, particularly the buccal portion.[16] It could be hypothesized that in restored premolars, the remaining tooth structure, toughness of material and presence of bonding could contribute to the fracture resistance of the restoration. Nevertheless, consideration on restorative materials also included being simple to use and less technical sensitive, particularly when concerning individuals with SHCN. A recent study has showed that ART using HVGI is an effective procedure to manage cavitated carious-dentine lesions in the disability.[1]

Renewal MI has been developed recently to match the concept of minimal invasive restoration, with simplifying the material handling while filling. The composite resin was available in an injectable capsule. While the adhesive step was omitted, the material could be applied into the prepared cavity using dispenser. Previous *in vitro* studies have showed that after a 28-days maturation in artificial saliva, the restoration was hygroscopic expanded and calcium/phosphate compounds were precipitated.[8] Microleakage test and Scanning Electron Microscopy (SEM) revealed a better sealing at both the enamel- and dentine-material interfaces. Without adhesive layer, the precipitation would become a reservoir for mineralization at the interface where dental tissues directly contacted the restoration.[8] It could be speculated that RMI might be a promising alternative restorative material for the demineralized dentin.

In present study, we examined the durability of RMI restoration in comparison with restorations using conventional materials, namely composite resin and HVGI. We also further compared whether additional of adhesive system would increase the fracture resistance of RMI. The MOD class II restoration in the premolar was chosen because the model would be the most vulnerable to post-operative failure.[17] Additional chemically-induced artificial caries would further challenged the durability. The chalky appearance and softened dentin on tactile exploration were noted. We, therefore, tested the strength of the restorations on the demineralized dentine. Subjecting the restoration under thermocycling at 5,000 and 10,000 cycles simulated approximately 6 to 12 months used in oral cavity[18] when the effectiveness of most restorations became noticeable clinically.

In this study, the double "apply-cure" application was chosen for composite resin and RMI restorations. Evidence has showed that this technic, using self-etched Universal adhesives, reinforced the quality and enhancing durability of the dentine bond[19] by which a thicker adhesive layer strengthening the mechanical properties of the resin-dentin interface, rather than enhancing the smear layer interaction.[20]

Fracture resistance test using in this study provide the highest mean fracture resistance on the sound-tooth group at approximately 1,500 N, which was in the range of recent studies.[21,22,23] In the negative control teeth filled with temporary material, IRM, the mean fracture resistance was at 700 N which slightly higher than those reported on no-fill-cavitated teeth (at approximately 500 N).[21,22,23]

Our findings indicated that the mean fracture resistance of the RMI, when applied conventionally without bonding, was not significantly different from composite resin and HVGI at both 5,000 and 10,000 thermo-cycles. When self-etched Universal bonding was added, there was also no significantly increase in the mean fracture resistance.

Comparison between 5,000 and 10,000 cycles showed no statistical difference in any restoration groups. It was interesting to noted that the fracture resistance of the no-bonding RMI slightly increased with the longer cycles ran. Additional step of self-etched Universal adhesive, which slightly had more fracture resistance force at 5,000 cycles, became slightly lower than those without bonding at 10,000 cycles. Previous study showed that increased interface precipitation was seen with aged RMI[8] which might be one of the reasons to explain the increasing fracture resistance at 10,000 cycles.

After selective removal, a large area of the cavity floor would have been caries-affected dentin.[24] Clinically, GI has been suggested as alternative due to its properties on chemical bonding to tooth structure, highly mineral discharge which induced remineralization at interface.[5] However, strength of the GI restoration in long-term might be compromised,[25] as showed by fracture resistance data at 10,000 cycle in this study. When composite resin was planned, adhesive systems must be carefully selected, in order to increase strength of the restoration in the cavity that caries-affected dentine was spared. It remained inconclusive that, in such compromised condition, which reagents and application methods would be the most compatible.[20,24] Nonetheless, clinical outcome at 3 yeas post-operatively showed that in multi-surface cavities, ART with GI faired a significantly better survival rate in SHCN patients, than those filled with conventional composite resin.[1] This was owing to the less technical sensitivity of GI restoration.

Material-science studies showed comparable physical properties of RMI to the available composite resin[7] which support its potential to be alternative to GI, for the ART restoration. Our comparisons at both thermocycling conditions showed that the GI restoration had lower, but not statically different, fracture resistance, than RMI groups. In this study, we proposed that the RMI might be used in 2 modes, with or without bonding. We hypothesized that when adhesive applied, the top-down remineralization from the RMI might be blocked but the mechanical strength at the dentine-

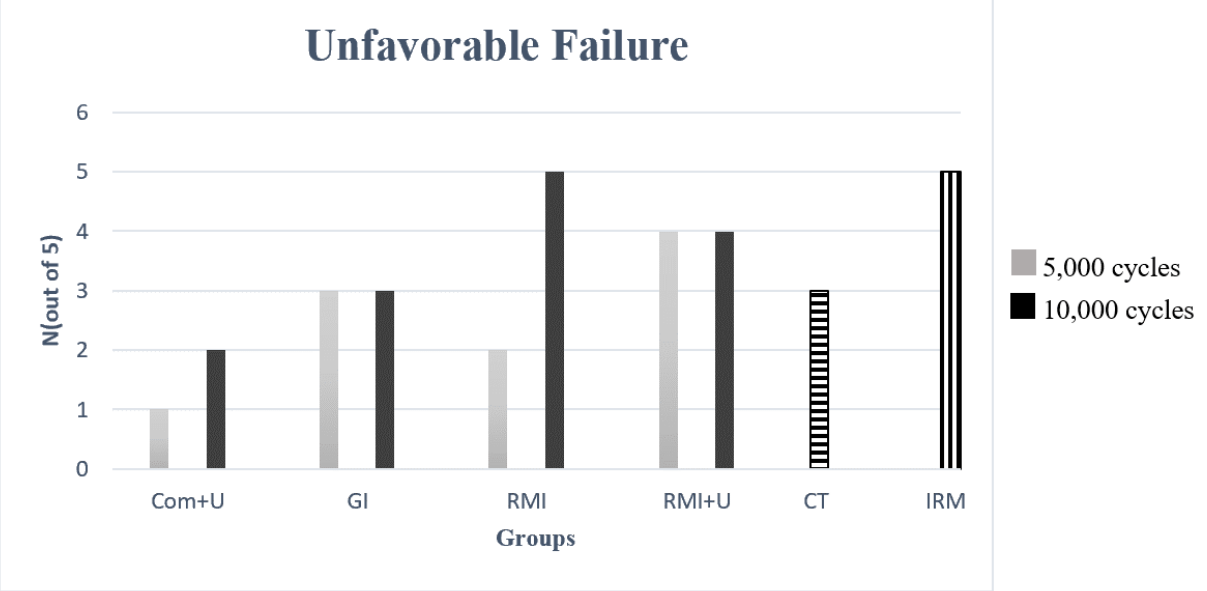

Figure 2. Shows distribution of unfavorable failure teeth (out of 5/group) in different restorative groups, at 5,000 and 10,000 thermocycles: horizontal line column represents failure in control group, vertical line column represents IRM group.

interface might improve the fracture resistance. Studies have showed that double application, similar to that used in this study, provided better stress distribution due to increased elasticity of the adhesive layer and improved degree of conversion due to enhanced curing.[20] Our findings, however, showed the opposite trends at 5,000 and 10,000 thermo-cycles. Further study to be tested on more sample numbers will clarify the preliminary results.

The inorganic filler loading in the nanofilled-composition; Filtek Z350XT was about 78.5% by weight.[26] The self-adhesive composite RMI contained the hybrid fillers loading at about 72% by weight, which had higher strength than that required by ISO 4049.[7] Besides silicate glasses, monocalcium phosphate (MCP), was another main filler added. In this study, the comparable mean fracture resistance of RMI and Filtek Z350XT could be attributed to these fillers. The UDMA monomer in RMI also improved in mechanical properties.[7,27] When considered the failure mode, despite higher fracture resistance force, higher number of unfavorable fracture was observed in both RMI groups, as compare to under 50% of the unfavorable teeth in composite resin groups. This supported by result from previous studies that nanofilled composite had better mechanical properties than microhybrid composite.[26,28,29]

As most studies were conducted on sound dentin, demineralized dentine might contribute to the unexpected results seen in this study. The pattern demonstrated in our study might be unable to imply on the complete caries removal cavity. Nonetheless, data obtained from this pilot study had been used for sample size calculation, for the ongoing study at 10,000 thermo-cycles condition.

Conclusion

All the restorative materials in this preliminary study endured the comparable fracture resistance when restored in the artificial caries, at the condition simulating 6 and 12 months in the oral cavity. However, teeth restored with composite resin using double application of self-etched mode Universal adhesive had the least unfavorable failure.

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OM-00019

Effectiveness of Intra-buccinator 8mg Dexamethasone Injection in Mandibular Third Molar Surgery

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ABSTRACT

Background and Objective: Extraction of mandibular third molar is one of the most frequently performed surgical procedure in oral surgery. Since there is no optimal standard protocol for anatomical location of dexamethasone administration in third molar surgery, high-quality randomized controlled trial is necessary to evaluate whether pre-operative intra-buccinator 8mg dexamethasone injection could reduce postoperative complications - facial swelling, pain, and trismus (limitation of mouth opening).

Methods: In our randomized controlled clinical study, we conducted split mouth design among twenty-one healthy participants who had symmetrical position impacted mandibular third molar bilaterally. Each side of participants was divided into group A (dexamethasone injection) and B (normal saline injection) as control group during their visits. Measurements for facial swelling and trismus in millimeters, pain using visual analog scale and number of analgesics taken, were assessed preoperatively (baseline), 3rd and 7th postoperative days. Statistical analysis using ANOVA, Wilcoxon, and independent t tests considered significant with $p<0.05$.

Results: Preoperative intra-buccinator 8mg dexamethasone injection significantly reduced postoperative facial swelling, trismus, and pain when compared to normal saline injection.

Conclusion: This is the first double-blinded clinical trial demonstrating the effectiveness of pre-operative intra-buccinator 8 mg dexamethasone injection in reducing postoperative facial swelling, pain, and trismus.

Keywords: Intra-buccinator dexamethasone injection, Postoperative facial swelling, Postoperative pain, Postoperative trismus, Third molar surgery

Proceeding

Introduction

Extraction of mandibular third molar is the most frequently performed surgical intervention in oral surgery.[1] Because surgical site has loose connective tissue and high vascularization, a variety of postoperative inflammatory responses are commonly anticipated.[2] Although inflammatory responses assist in wound healing, excess inflammation induces postoperative complications including facial swelling, pain and trismus.[3]

Postoperative facial swelling is caused by exudate accumulation in surrounding tissue from inflammation-induced vascular events including vasodilation, increased vascular permeability, and changes in local hydrostatic and osmotic pressures.[4] It is additionally exacerbated by interstitial fluid accumulation from lymphatic obstruction by fibrinogen clots of damaged vasculatures.[5] Additionally, in response to surgery related tissue damage, inflammatory cells and surrounding vasculature release arachidonic acid mediators - prostaglandin, 5HT, and bradykinin which increase exudation, evoke pressure on nerve terminals and cause postoperative pain.[6] These mediators trigger postoperative pain by intensifying local nociceptors responsiveness and postoperative trismus is induced by inflammation and exudate accumulation within surrounding muscles and fascia.[5,7] Trismus is also commonly occurred due to facial swelling and pain associated with surgical trauma.[8]

Anti-inflammatory corticosteroid therapy significantly reduces postoperative inflammation and its complications by inhibiting arachidonic acid mediators release.[9] Dexamethasone is corticosteroid commonly used in oral surgery because of its long-acting, extremely potent, lower sodium retention, and pure glucocorticoid effects.[10,11] Administering 8mg dexamethasone from different routes produced comparable clinical effectiveness for controlling post-operative pain, facial swelling, and trismus in molar surgery.[12,13,14,15,16,17] However, as there is no optimized standard procedure for dexamethasone administration, clinical trials are further required to provide quality evidence of dexamethasone administration strategy.

Buccinator muscle is flattened, square-shaped muscle that runs parallel to major axis of mouth and forms muscular wall of anesthetic triangle.[18] Anatomical landmarks such as buccal and lingual spaces including buccinator muscle were remarked as the frequency of postoperative swelling after impacted third molar surgery.[19] Due to its location in anesthetic triangle and proximity to third molar surgical site, dexamethasone could be injected painlessly into buccinator muscle after local anesthesia. This suggests a potential alternative injection site of dexamethasone in controlling postoperative complications. No study has conducted to investigate

effectiveness of pre-operative intra-buccinator 8mg dexamethasone injection in molar surgery. The primary objective of this study was to assess the effectiveness of intra-buccinator 8 mg dexamethasone injection for alleviating postoperative pain, facial swelling and trismus in third molar surgery using a double-blinded, randomized controlled clinical trial with split mouth study design. The null hypothesis stated that there is no difference in pain, swelling and trismus between dexamethasone and normal saline injection at mandibular third molar surgery.

Materials and Methods

Study Design and Randomization

This study was performed according to approval of committee of human rights to human experimentation at Mahidol University (protocol number 2020/DT004). This study was approved by the Research Ethics Committee and the Institutional Review Board of Faculty of Dentistry with COA number MU-DT/PY-IRB 2020/048.0309 and registered in Thai Clinical Trials Registry (TCTR20210623001).

A double-blinded, randomized, controlled clinical trial using split mouth study design was conducted at Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Mahidol University, Bangkok from November 2020 to July 2021. The sample size was calculated based on the data extracted from previous study at 95% confidence level.[20] A total number of twenty one participants without underlying medical diseases between 18-45 years with symmetrically bilateral impacted third molars were enrolled and performed by same surgeon. Participants with a history of acute infection, allergies to corticosteroids, antibiotics, or local anesthesia, history of using opioid analgesic or antidepressants, alcohol abuse or narcotic use, refusal or failure to follow postoperative instructions, pregnancy and lactation were excluded. To remove inter-subject variabilities including pain threshold, anxiety, and lifestyle habits and to use each participant as his or her own control to minimally influence treatment effects, a split-mouth study design was used. In this study, each participant's mouth was divided into two segments to randomly assign into group A (dexamethasone injection) and B (normal saline injection). All participants were randomly assigned to group A or B during their first visits using a computer-generated number sequence and then allocated to another group to receive different treatment at their subsequent visit. The envelope containing computer-generated numbers to assign participants to different groups was sealed by designated person. Since this study is double blinded, the assigned dentist or assistant allocated participants and prepared dexamethasone or normal saline according to the information written in sealed envelopes. Until the end of the study, these envelopes were kept secure. The entire procedure of randomizing and assigning participants, as well as treatment they received in their visits, was double-blinded to both participant and surgeon.

Surgical Procedure, Medications and Assessments

The surgeon performed preoperative assessments on all participants including history taking, clinical examination, preoperative measurements, and orthopantomogram. The objectives, potential complications, postoperative instructions were explained to participants and informed consent were obtained. The participant was given preoperatively 2ml of 8mg of dexamethasone or normal saline by using surgical syringe with disposable hypodermic 27 gauge needle immediately after local anesthetic effect. Anatomical landmark for buccinator muscle is into the mucous membrane opposite to first molar and 3-4mm inferior to the Stensens' duct. The same surgeon performed two molar extractions in each participant by four weeks apart. Total duration of operation from initial incision until suturing was recorded. After providing antibiotics, opioid analgesic as rescue medicine and postoperative instructions, participants were then evaluated on 3rd and 7th postoperative days.

Facial swelling was measured with a tape using a three-point analysis including Corner of eye-Angle of mandible (Ex-Go), Tragus-Commissure of mouth (Tr-Ch), and Tragus-Pogonion (Tr-Pg). Mouth opening for trismus was deliberated in millimeters between incisal edges of upper and lower central incisors using a vernier caliper.

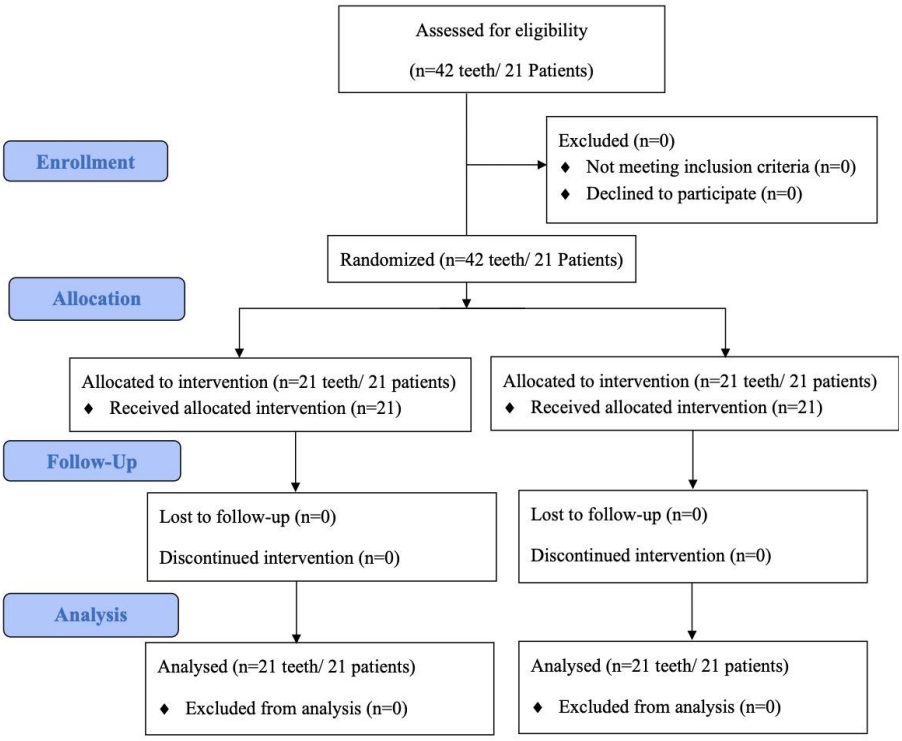

Figure 1. CONSORT flow diagram of this study.

Participants were instructed to self-assess and record postoperative pain using visual analog scale (VAS) with "no pain" (0-4 mm), "mild pain" (5-44 mm), "moderate pain" (45-74 mm), and "unbearable pain" (75-100 mm), as well as amount of analgesic consumption was documented for one week. Clinical assessments of facial swelling, trismus and pain were conducted preoperatively (baseline), 3rd and 7th postoperative days by the same surgeon. The surgeon filled out the proforma using the data from all participants.

Statistical Analysis

Data analysis was accomplished using Statistical Package for Social Sciences (SPSS version 17.0, IBM Corp) and included descriptive statistics, and factorial repeated measure ANOVA with Bonferroni adjustment for multiple comparisons to assess mean difference in various parameters between groups. The significance of differences between groups was assessed using independent t-test. $p<0.05$ was considered as statistically significant.

Results

In this study, each mouth of 21 healthy volunteers between 18-45 years who did not meet any exclusion criteria were divided into two segments to randomly assign into group A (dexamethasone injection) and B (normal saline injection), as shown in the CONSORT (CONsolidated Standards for Reporting Trials) flow diagram (Fig. 1). According to Pell and Gregory's classification, class II position of third molar impaction was observed in 90.50%, which was followed by class I position (9.52%). Position A impaction affected 47.61%, while position C affected 14.28% of participants. According to Winter classification, mesio-angular and horizontal impaction each accounted for 43%, followed by disto-angular (9%) and vertical (5%) impactions. The operation time with dexamethasone injection was 40.95±5.95 minutes, and 39.19±8.87 minutes for normal saline injection with no significant difference in operative durations between groups.

In comparison to normal saline injection, measurements of facial swelling including Ex-Go, Tr-Ch, and Tr-Pg were significantly reduced on 3rd postoperative day when the participants received dexamethasone injection ($p<0.05$) (Table 1) (Fig. 2A-C). There was significant improvement in mouth opening on 3rd and 7th postoperative days with dexamethasone injection as compared to normal saline injection ($p<0.05$) (Table 1) (Fig. 2D). According to these collected data, intra-buccinator dexamethasone injection produced significant effectiveness in reducing facial swelling and trismus as compared to normal saline injection.

Table 1. Comparison of facial swelling and mouth opening between dexamethasone and normal saline injection.

Parameters	Evaluation Day	Mean (SD)		*p*-value
		Dexamethasone	Normal Saline	
Ex-Go	Preoperative baseline	105.90 (6.92)	105.77 (6.69)	0.951
	3rd postoperative day	110.09 (7.76)	116.22 (11.19)	0.046*
	7th postoperative day	108.14 (6.55)	111.33 (7.46)	0.149
Tr-Ch	Preoperative baseline	121.33 (7.98)	120.17 (8.24)	0.647
	3rd postoperative day	125.52 (8.18)	132.10 (9.62)	<0.001*
	7th postoperative day	123.52 (7.76)	125.09 (7.44)	0.507
Tr-Pg	Preoperative baseline	153.61 (12.45)	154.09 (10.37)	0.894
	3rd postoperative day	157.61 (12.48)	164.04 (11.24)	<0.001*
	7th postoperative day	154.73 (12.39)	159.48 (9.08)	0.670
Mouth Opening	Preoperative baseline	38.80 (6.35)	38.96 (4.21)	0.921
	3rd postoperative day	36.32 (4.83)	30.44 (4.48)	<0.001*
	7th postoperative day	36.73 (5.3)	34.57 (4.62)	<0.05*

Independent t test, *Significant relationship ($p<0.05$) ($p<0.001$)

Participants also reported lower pain scores throughout the evaluation period from postoperative day 1 to day 5 when they received dexamethasone injection ($p<0.05$), in comparison to normal saline injection (Table 2) (Fig. 3). The average number of analgesics consumed was significantly lower when participants received dexamethasone injection as compared to normal saline injection ($p<0.001$) (Table 2). There were no adverse events, accidents, or pre-, trans-, or postoperative complications, after intra-buccinator injection of dexamethasone or normal saline in this study.

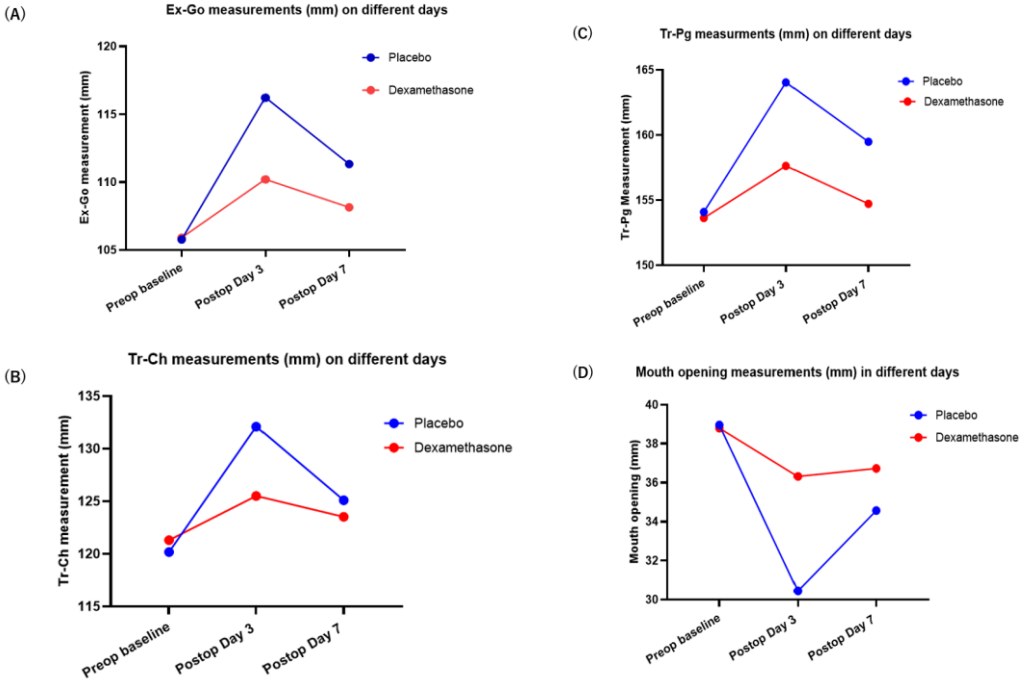

Figure 2. Distribution of estimated means of swelling in terms of (A) Ex-Go, (B) Tr-Ch, (C) Tr-Pg measurements, (D) limitation of mouth opening compared between dexamethasone and normal saline injections on different days.

Table 2. Comparison of mean VAS pain scores between dexamethasone and normal saline injections on different postoperative days.

Evaluation Day	Mean (SD)		*p*-value
	Dexamethasone	Normal Saline	
1st postoperative day	34.85 (15.76)	63.38 (11.25)	<0.001*
2nd postoperative day	21.42 (14.35)	43.42 (19.18)	<0.001*
3rd postoperative day	14.33(11.11)	31.57 (17.77)	0.001*
4th postoperative day	7.52 (7.31)	17.71 (11.45)	0.001*
5th postoperative day	3.95 (4.49)	10.71 (8.85)	0.003*
6th postoperative day	2.33 (5.64)	4.57 (4.39)	0.159
7th postoperative day	1.52 (2.92)	4.00 (4.78)	0.050

Independent t test, *Significant relationship (p<0.05)

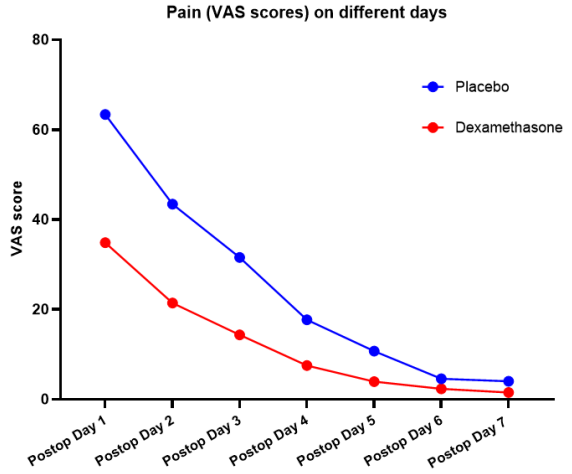

Figure 3. Distribution of estimated means of pain between dexamethasone and normal saline injection on different days.

Discussion

Extraction of mandibular third molar is the most frequently performed surgical intervention in oral surgery.[1] Inflammatory response is unavoidable consequences since surgical site is highly vascularized and has loose connective tissues.[2] Although these inflammatory responses assist in wounds healing, excess inflammation contributes to serious postoperative complications - facial swelling, pain and trismus or limitation in mouth opening.[3] Various formulations of 8mg dexamethasone corticosteroid therapy were prescribed in the form of tablets, injectable solutions, intravenous or extraoral or intraoral muscular administrations and it has been shown effective in lowering postoperative facial swelling and pain in molar surgery by suppressing inflammatory mediators release and transudate exudation.[10,11] However, preoperative intra-buccinator 8mg dexamethasone injection has never been studied for its effectiveness in third molar surgery. The current study demonstrated that pain, swelling and trismus was significantly reduced in dexamethasone injection site compared to that of normal saline injection site. Therefore, the null hypothesis is rejected.

The participants in this study mostly had bilaterally symmetrical impacted teeth and operated by same surgeon with no significant differences in surgical duration. It indicated that there was no presence of variables that influence postoperative inflammatory responses and complications.[21] Previous study also used a split-mouth study design to remove inter-subject variabilities including pain threshold, anxiety and lifestyle habits.[22] Our study design also used each participant as his or her own control which minimally affected treatment effects. In our study, neither intra-buccinator dexamethasone nor normal saline injection produced adverse effects.

Postoperative facial swelling in third molar surgery is caused primarily by exudate accumulation in surrounding tissue due to increased vascular permeability, changes in local hydrostatic and osmotic pressures, as well as lymphatic drainage obstruction.[4,7] At different postoperative days in this study, all means of Ex-Go, Tr-Ch, and Tr-Pg measurements for facial swelling were significantly lower on 3rd postoperative day when the participants received preoperative intra-buccinator 8mg dexamethasone injection, as compared to normal saline injection in their opposite sites. These findings were comparable with previous trials which also yielded a significant reduction in postoperative facial swelling by dexamethasone injection into masseter muscle either before or after surgery.[15,23,34]

Trismus or limited mouth opening is a consequence of surrounding muscles inflammation and exudate accumulation within fascia, which lowers the quality of life by making eating and speaking difficult.[4,7] In this study, trismus was less pronounced significantly on 3rd and 7th postoperative days when the participants received dexamethasone injection, as compared to normal saline injection in different site. Although corticosteroids have no direct effects on muscle contraction, anti-inflammatory effects of dexamethasone for lowering exudation and oedema could secondarily decrease postoperative trismus when participants received dexamethasone injection.[25] These finding were in agreements with previous studies that reported significant reductions in postoperative trismus using intra-masseter dexamethasone injection or localized application of dexamethasone powder.[15,24,26] In contrast, the earlier study which discovered no significant difference in trismus reduction with the same dosage of dexamethasone administration through oral route.[27] Previous study, Bortoluzzi et al[27] declared there is no significance difference for trismus but on the other hand, Autune et al[14] approved 8mg dexamethasone is effective for reduction of post operative swelling, pain and trismus. There is controversial for dose, route and site of anatomic location for dexamethasone administration as standard protocol and our study showed the favorable result of 8mg intra buccinator dexamethasone.

The inflammation from tissue injury induces postoperative pain after third molar extraction.[4,7] According to pain assessment using self-reported VAS score, there was a statistically significant lower pain score on different postoperative days when the participants were given dexamethasone injection as compared to their normal saline injection. The average number of analgesic tablets consumed on different postoperative days was significantly lower with dexamethasone injection as compared to normal saline injection in each participant. These finding were comparable to the outcomes of several studies which demonstrated a significant decrease in postoperative pain and analgesics requirements with administration of dexamethasone from different routes intramuscular, submucosal, and local powder.[20,28,29] According to previous comprehensive analysis, preoperative intramuscular dexamethasone injection was significantly more effective than other NSAIDs and methylprednisolone in reducing postoperative pain in third molar surgery.[30] In prior studies, however, intramuscular or submucosal dexamethasone administration did not provide any improvement in postoperative pain of third molar surgery, which was contrary to the findings of our study.[15,27]

Our study has some limitations including the use of smaller samples and single center randomized study. Because the different studies around the world discovered varying benefits of dexamethasone via different routes in controlling postoperative inflammatory complications, future research using larger sample sizes with multi-center randomized design, different doses and comparative routes of dexamethasone will be needed to better understand the clinical significance of intra-buccinator

dexamethasone injection for postoperative facial swelling, trismus, and pain.

Conclusion

This is the first double-blinded randomized controlled clinical trial which demonstrated that pre-operative intra-buccinator 8 mg dexamethasone injection was a promising alternative strategy for minimizing postoperative facial swelling, pain, and trismus in third molar surgery, with the added benefits of easier administration and localized anti-inflammatory effects at surgical site.

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Oral Health Care Service in Nursing Homes in Bangkok

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ABSTRACT

Background and Objective: A nursing home is a long-term care facility for older adults or dependents with health rehabilitation, supporting and promoting activities with accommodation. Oral health is highly correlated to the status of overall health and quality of life. This study aimed to assess oral health care services in nursing homes in Bangkok.

Methods: This study was a cross-sectional study in nursing homes in Bangkok, Thailand (N=20). The informed consent was obtained from respondents before administering the questionnaire by phone call. The questions included demographic data, health care service and oral health care service of those nursing homes.

Results: Nursing homes in Bangkok have various types of services. All nursing homes provide daily oral care and denture care to their residents. Denture care involved brushing and immersing in solution or tap water. Denture cleansing tablets were used in only 20% of nursing homes. Dental checkups were only performed in the case of an emergency, and only 35% of nursing homes offered additional services with a cost for visiting dentists.

Conclusion: Nursing homes in Bangkok provide oral health care and denture care for their residents. There were no guidelines or protocols for ruling out the problems. The dentist should play an important role in suggesting the nursing home caregivers for oral health care and evaluating oral health problems in their residents.

Keywords: Dental checkup, Denture care, Long-term care facilities, Nursing home, Oral health care

Introduction

There are more older adults increasing at an accelerated pace, especially in developing countries. The structural change in the older adults of the global population is becoming one of the main challenges that affect several sectors, such as health and social care. The demand for primary health and long-term care also increases.[1] Thailand is a developing country that is facing the most rapid rate of population aging. This impacts social security, health care expense and equity. These problems need proper National policy and management for an aging population in long-term care facilities (LTCFs). A more significant proportion of older adults in Thailand have had a higher level of education. The living of older adults has changed from living with children and family to living alone or with only the spouse. Older adults with fewer children are less likely to live with an adult child, suggesting that the trend toward smaller families, combined with the greater dispersion of children, will contribute to a continuing decline of co-residence with children.[2] According to the Department of Older Persons, older adults accounted for 17.57 percent of the population on December 31, 2020. One-fifth of the people in Bangkok was older adults (19.83%), or almost 10% of the total population in Thailand.[3] Therefore, this study focuses on nursing homes in Bangkok, the metropolis with the highest proportion of older adults and the highest number of nursing homes in Thailand.[4,5]

Long-term care facilities (LTCFs) can also help reduce the inappropriate use of acute healthcare services and help families avoid catastrophic care expenditures.[6] Similarly, the prevalence of oral problems increases with age.[7] As people live longer with their natural teeth, the complexity and problems of their oral health concerns arise.[8] Poor oral health can have severe consequences in physical conditions or problems for older adults such as respiratory infections,[9] cardiovascular diseases,[10] and impaired nutritional health. Poor oral health can also result in a loss in the quality of life for older adults,[11] who may suffer from low self-esteem and social isolation.[12]

Thailand's Ministry of Public Health regulates all long-term care facilities. The Ministry of Public Health announced "Ministerial Regulations on Care for the Elderly or Dependents" on July 20, 2020 that separate those facilities from other healthcare facilities. LTCFs for older adults and dependents must register for the license in 3 types; Daycare, Independent living and Nursing homes. This Ministry Regulation regulates three main standards for the LTCFs: construction, safety, and service standards.[13] Nursing homes require more criteria and regulations than other types of LTCFs, especially in terms of the annual fee and those three standards. These make the annual fee twice that of daycare. For the safety standards, it requires resuscitation equipment and defibrillator. The service standards, it requires multidisciplinary rehabilitation to promote health. The service standards cause a minimum caregiver-to-resident ratio to 1:5.[14]

There is the Announcement of the Board of Health Enterprises for criteria for testing and evaluating of knowledge and competencies of persons taking care of the elderly or people with dependency B. E. 2020. These regulations require the LTCFs' operators to be licensed prior open for service.[15] Also, the persons eligible to take the examination for a license must first pass the "130-hour Elderly or Dependency Care Operator" course or have graduated from within five health-field sciences courses[15] as shown in the result.

The health and oral health services in nursing homes are influenced by several factors such as the operator's occupation, caregivers' skill, monthly expenses, etc. The physical health activities, nutritional concerns, or regular health checkup service are all aspects of health care service in each nursing home. The oral health care activities including frequency of oral care routine, denture care, and dental checkup are differed from each LTCFs. The information of characteristics, health care service and oral health care service were collected in this study. This study aimed to assess oral health care services in nursing homes in Bangkok.

Materials and Methods

Participants and study design

This study was a cross-sectional study in nursing homes in Bangkok, Thailand. The participants were licensed by the Department of Health Service Support, a government organization in Bangkok, before 7 March 2022.

After the announcement of the "Ministerial Regulations on Care for the Elderly or Dependents on July 20, 2020, up to March 7, 2022, there were 137 nursing homes registered with the Department of Health Service Support.[4] The names of the fifty nursing homes were randomly selected and then were made a phone call until May 2022. Those 20 nursing homes that allowed to give information were informed consent prior to the administration of the questionnaire. The respondents were owners, operators or caregivers in each nursing home.

The study was approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (IRB No: HREC-DCU 2021-103).

Questionnaire and data collection

The questionnaire included about characteristics of nursing homes including average age of their residents, the number of service capacity, and the resident' monthly expense in those nursing homes. The following section contained health care service including regular health checkups in nursing homes, physical health activities and nutritional concerns. In part of oral health care service, there were questions of routine oral health care, denture care and dental checkups.

All data on demographics, health care service and oral health care service in nursing homes were presented in descriptive analysis.

Results

There are three types of LTCFs, nursing home is the one which had been most registered for the Department of Health Service Support. In this study, due to Covid Pandemic, all of the selected nursing homes provided information in phone call survey. There were 20 nursing homes participated in this study followed the Ministerial Regulation. Table 1 showed all characteristics of the nursing homes participated in this study. As regulation, long-term care facilities' operators have licensed the assessment on the operator's competence in caring for the Elderly or Dependent, B.E. 2020. Considering about the operator of the nursing home, 45% of them have gotten the Master of Nursing Science Program. Follow by 40% had taken a 130-hour Elderly or Dependency Care Operator course.

The average age of resident in samples mainly was lower than 65 years (40%). There were 35% and 20% of the total samples reported that they had an average age of 66 – 70 years and more than 76 years, respectively. In the service capacity, half of nursing homes had 11 – 30 beds in service, followed by 20% of the samples that had less than 11 beds and others that had more than 51 beds in service. The service expense per month were also varied. Most of the nursing homes cost 15K – 19K THB per month while the remaining cost more than 19K THB.

Table 1. Characteristics of nursing homes in Bangkok.

Characteristics	N	(%)
Nursing Home Operator		
Doctor of Medicine Program	2	(10%)
Doctor of Dental Surgery Program	0	
Bachelor of Public Health	1	(5%)
Bachelor of Science Program in Physical Therapy	0	
Master of Nursing Science Program	9	(45%)
130-hour Elderly or Dependency Care Operator course	8	(40%)
Average age: years		
≤ 65	8	(40%)
66-70	7	(35%)
71-75	1	(5%)
≥ 76	4	(20%)
Service capacity: beds		
≤ 10	4	(20%)
11-30	10	(50%)
31-50	2	(10%)
51-70	4	(20%)
Service expense per month: THB		
15K-19K	12	(60%)
19K-22K	5	(25%)
22K-25K	2	(10%)
> 25K	1	(5%)

Table 2 showed nursing homes health care services, all of the residents in nursing homes had regular health checkup by their physician appointments. They also had physical health activities (such as aerobics, dancing or drawing) to maintain the health condition of residents and nutritional concerns individually for health and oral health of each resident. From the phone call survey, we found that 60% of nursing homes offered additional services for a cost to residents who needed routine checkups by nursing home staff, while the remaining attend checkups with resident's families.

Table 2. Health care service in the survey nursing homes.

Health Care Service	N	(%)
Health checkup		
Regular/Patient appointment	20	(100%)
Physician visit		
By Resident's family	8	(40%)
By Nursing home staff (additional cost)	12	(60%)
Physical activity and Nutritional concern		
Yes	20	(100%)

Table 3. Oral health care service in the survey nursing homes.

Oral Health Care Service	N	(%)
Routine oral and denture care		
Daily	20	(100%)
Denture immerse		
Immerse with tap water	16	(80%)
Immerse with Denture cleansing tablet	4	(20%)
Dental checkup		
Emergency/Chief complaint	20	(100%)
Dentist visit		
By Resident's family	13	(65%)
By Nursing home staff (additional cost)	7	(35%)

The oral health care services of nursing homes were shown in Table 3. It was found that all nursing homes have provided daily oral health care for their residents. They also concern about wearing dentures in residents and denture care in daily oral care. 20% of nursing homes use denture cleansing tablets to all residents with dentures, while 80% immersed in tap water except some cases that the families bring for them. For dental examination, it is found that dental check-ups will be provided for residents only when they have oral health problems. In the case of dental visits, this study discovered that 35% of nursing homes offer a service to transport their residents to dentists while the others wait for their family's support.

Discussion

There were 137 nursing homes licensed by the Department of Health Service Support before 7 March 2022. In this study, 20 nursing homes in Bangkok consented to respond to the questionnaire through phone calls. The Ministerial Regulations force that all nursing homes prepare for life support and rehabilitating health. However, there was no details to control about oral health care service in LTCFs. In terms of the operator, there is a requirement that graduates from all 5 health science fields can be evaluated for a license to operate in LTCFs. For those who have not got theses degrees, the other choice is to be trained in a 130-hour Elderly or Dependency Care Operator course prior to taking the license. It is essential that those who graduate in health sciences have adequate understanding of health care to obtain a licensed without additional training. This should make a difference in health services in LTCFs that need further study.

The study revealed that younger people showed a larger portion of the average age group. It demonstrates that services in nursing home are varied and not limited to the older adults or dependents. The information obtained from the interview also revealed that there are people who prefer to be cure after surgery or while illness, these need rehabilitation for good quality of life. As a result, it was residents who were 65 and younger in nursing homes. Nursing homes in this study varied from small capacity (≤ 10 beds) to large capacity (51 – 70 beds). Service expenses per month also range from 15K – 19K THB to more than 25K THB which relate to variety of services in nursing homes. According to the study, the service expense of nursing homes is relatively high compared to Thai people's monthly income (THB27,352 in 2021).[16] Therefore the total family's monthly income must be enough to support family members who are expected to reside in a nursing home for a long time period.

In terms of health care service, residents in nursing home have high levels of physical dependency, cognitive impairment, multiple morbidity and polypharmacy.[17] From this study, nursing homes in Bangkok focused on and administered the physical health of residents by regular health checkup with their physician. There was a service in some nursing homes to take the residents to see physician with an additional cost, which accounts for 60% of all nursing homes. In 40%, resident' family have to take responsibility to take them for regular checkup. Physical activity positively enhances cognitive performance, executive functions, physical endurance, and depressive mood[18] in all patients with dementia in Nursing home, indicating that these interventions may serve as a cost-effective and accessible therapy in addition to or in place of pharmacotherapy.[19] Malnutrition, weight loss, and low BMI are prevalent concerns in long-term care facilities. Low BMI and weight loss have been related to increased mortality.[20] This study found that all nursing homes provided activities for residents. There were a variety of physical activities such as walking, dancing, drawing, etc. All nursing homes provided nutritional concern with physical health and oral health for each resident. It needs further study for details on the causes and effects of physical activities and nutritional concerns in nursing homes.

In this study, oral health care is provided to residents daily, while denture care for those who wear dentures was done by brushing and immersing as a routine. According to the study in USA[21], the dependent older adults in a nursing home should practice daily oral health care and receive routine dental checkups by dentist. Education of nursing home caregivers and older adults themselves about the significance and procedures of oral health care is essential.[21]

The study of Y Nishi et al said that number of microorganisms on the surface of a removable denture can be reduced by brushing and cleaning with denture cleanser.[22] The study of L Gendreauhe and Loewy ZG found that poor denture cleanliness is a risk factor for dentures stomatitis, and there is a direct correlation between denture cleanliness and a higher risk of and prevalence of denture stomatitis.[23] The researches support that denture cleansing tablet is significant reduction in candida count and bacterial load in denture surface. [24,25] In this study, there are two types of immersing which are immersing with tap water in mainly and with denture cleansing tablet. The denture cleansing tablet accounted for 20% of all sample, while the tap water accounted for 80%, expect for some residents that their family bringing other solutions or tablets for them. It would be beneficial to have a set of guidelines or standards for oral health care service in nursing homes.

In regards to dental care, the dental checkup (with dentist) was found only if there was a problem or emergency case, and 65% of sample nursing homes were informing family or relatives to take them to the dentist, with only 7% providing services to take residents to dentist with an additional cost. These results correspond with the results of the German study.[26] The older adults in nursing homes do not attain routine dental checkups; they can only visit the dentist if they are unpleasant or have oral health problems. The oral health is frequently one of the first parts of the body ignored by people with chronic disease and age-related impairments.[21] Regular dental checkups are suitable for early detection of oral problems, leading to appropriate treatment and cost savings. The dentist must actively create and maintain oral health care services and procedures in LTCFs. This study agrees with the recommendations that provided to help operators and caregivers establish oral health care protocol or guideline for their LTCFs.[27]

Due to the available evidence, data must be gathered via telephone survey. The collected data might not be entirely realistic. The phone call survey introduced the possibility of selection bias than on-site recruitment whether it is actual number of beds or caregiver ratios. In addition to providing information over the phone, people in various positions impact the relevant information. For instance, a caregiver at a nursing home will provide more probable information about health care and oral health care services than other staffs.

Conclusion

Nursing homes in Bangkok provide oral health care and denture care for their residents. There were no guidelines or protocols for ruling out the problems. The dentist should play an important role in suggesting the nursing home caregivers for oral health care and evaluating oral health problems in their residents.

Acknowledgements

This study used data from the nursing homes licensed by the Department of Health Services Support, a government organization in Bangkok.

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OM-00022

Anti-FimA Antiserum Increases *Porphyromonas gingivalis* Invasion into H357 Cells

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ABSTRACT

Background and Objective: *Porphyromonas gingivalis* (*P. gingivalis*) is a pathogen causing periodontitis. The pathogen exhibits abilities to induce inflammation, tissue damage, and it can invade host cells. Fimbria is a protein structure playing a role in cell attachment which will enable the bacteria to invade host cells. In this study we aimed to investigate the effect of antiserum against FimA, a subunit of fimbria, on invasion of *P. gingivalis* into H357 cells.

Methods: FimA protein was produced in *Escherichia coli* and purified using a nickel column. Two rabbits were immunized with the purified FimA protein. Anti-FimA anibody levels in the rabbit sera were determined by ELISA. To investigate the effect of the antiserum on *P. gingivalis* invasion, the bacteria were pre-incubated with the rabbit serum before co-incubation with H357 cells. The number of *P. gingivalis* invaded into the cells was counted after cells were treated with metronidazole, lysed with water, plated on blood agar and incubated at 37°C in anaerobic condition.

Results: After immunization with FimA protein, rabbit sera contained high levels of anti-FimA antibody as determined by ELISA. Surprisingly, numbers of bacteria inside H357 host cells were higher when the bacteria were pre-incubated with anti-FimA antisera compared to those pre-incubated with pre-immune antisera.

Conclusion: Incubation of anti-FimA sera with *P. gingivalis* enhanced host cell internaization of the bacteria.

Keywords: Antibody, Cell invasion, FimA protein, H357 cell, *Porphyromonas gingivalis*

Introduction

P. gingivalis is a non-motile asaccharolytic gram-negative rod anaerobic bacterium causing periodontitis.[1,2] *P. gingivalis* express many virulent factors, such as cysteine proteinases (gingipains), lipopolysaccharide (LPS), and fimbriae. *P. gingivalis* fimbriae play a role in adherence to salivary proteins, extracellular matrix, host cells and other bacteria. Thus it can attach and colonize in the oral cavity especially in gingival sulcus. *P. gingivalis* fimbriae present in 2 forms, major (FimA) and minor fimbriae.[2-4] Many researchers have developed many vaccines targeting fimbriae. In FimA vaccine studies, the vaccines could induce specific salivary IgA and serum IgG and also protected mice from alveolar bone loss inducing by *P. gingivalis*.[5,6]

It was expected that antibody against *P. gingivalis* FimA could inhibit its pathogenicity by inhibiting bacterial adhesion to host cells and hence preventing them from host cell invasion. The rice cell-produced mouse monoclonal anti-FimA antibody inhibited *P. gingivalis* invasion of human papilloma KB epithelial cells and human periodontal ligament cells.[7,8] Pre-incubation of *P. gingivalis* with mouse anti-FimA monoclonal antibody leaded to reduction of periodontitis and arthritis in collagen-induced arthritis (CIA) mice. In addition, FimA Ab attenuated bacterial attachment and aggregation on human gingival and rheumatoid arthritis synovial fibroblasts.[9]

In this study we preliminary investigated the effect of rabbit anti-FimA antiserum effect on *P. gingivalis* invasion into H357 cells. H357 is a tongue squamous cell carcinoma which has been used as a host cell model in *P. gingivalis* host cell invasion assays.[10] It was expected that the rabbit anti-FimA antiserum would inhibit *P. gingivalis* invasion. However, the result was contrary to this expectation.

Materials and Methods

FimA and anti-FimA antiserum productions

Histidine-tagged FimA typeI (His-FimA) was amplified with Thermo Scientific Phusion Hot Start II High-Fidelity DNA Polymerase (Thermo Scientific, IL, USA) from pcDNA3.FimA-PVXCP,[11] using forward primer incorporating *Hin*dIII site followed by enterokinase site at the 5' end, and reverse primer containing *Hin*dIII site at the 5' end. All primers were ordered from Pacific Science Co., Ltd., Bangkok, Thailand. The PCR product sequence was *Hin*dIII site, enterokinase site, FimA type I, and *Hin*dIII site, respectively. The gel purified PCR product was inserted into *Hin*dIII pre-digested pET28a plasmid. The ligation product was transformed into JM109 and the transformed cells grew on LB plate containing 50 □g/ml of kanamycin was confirmed by PCR and DNA sequencing.

The recombinant plasmid was purified and transformed into BL21DE3 *E. coli* for histidine tagged FimA (His-FimA) protein expression. His-FimA was purified using Nickel column (Thermo Scientific) and histidine tag was removed by enterokinase enzyme (Bio Basic Inc, Markham Ontario, Canada). FimA protein was administered intravenously (i.v.) to 2 female New Zealand White rabbits (Nomura Siam International Co.,Ltd., Bangkok, Thailand). The animal experiment was approved by Naresuan University

Institutional Animal Care and Use Committee (project no. NU-AE600814). FimA protein was diluted to 200 µg in 0.5 ml sterile saline solution then administered i.v. with a 25-gauge, 1-inch needle in marginal ear vein. FimA boosting was done on day 14, 28 and 42 with 100 µg FimA protein diluted in 0.5 ml sterile saline solution. Rabbit blood samples were collected from marginal ear vein at day0 for pre-immune serum and day49 for immunized serum. The final blood collection and euthanasia were conducted on day55.

Antibody analysis

Blood samples were allowed to clot at room temperature for 1 h followed by centrifugation at 2500 rpm for 15 min at room temperature. Serum was transferred to a new tube and kept at -20°C until use. To evaluate the amount of anti-FimA or anti-*P. gingivalis* IgG in blood, sera were analyzed by enzyme-linked immunoassay (ELISA) using 1 µg/ml of FimA protein or formalin-fixed *P. gingivalis* ATCC33277 suspensions which were diluted to an optical density of 1.0. The antigens were coated on 96-well plate overnight at 4°C. After incubation with serially diluted serum, the amount of bound IgG was detected by peroxidase-conjugated anti-rabbit IgG (Cell Signaling Technology, Massachusetts, USA) followed by the addition of O-phenylenediamine substrate (Thermo Scientific). The OD490 was recorded using xMark™ microplate spectrophotometer (BIO-RAD, California, USA).

H357 invasion assay

The human cell culture experiments were approved by the Naresuan University Research Ethics Committee Approval (IRB No. 590/59). H357 cells (Sigma-Aldrich, Missouri, USA) were cultured in DMEM:HAMS F12 (1:1) (Gibco, Thermo Fisher Scientific) supplemented with 2mM Glutamine (Gibco), 10% Foetal Bovine Serum (Hyclone Laboratories Inc, Utah, USA) and 0.5 µg/ml sodium hydrocortisone succinate (Sigma-Aldrich). H357 was seeded into 24-well plates at 5 x 10^4 cells per well 24 hour prior to invasion assay. The adherent cells were washed three times with PBS before addition of 2% (w/v) BSA (BIO-RAD) in serum free media and incubation for 1 h at 37°C to block the non-specific binding sites for bacteria. *P. gingivalis* was pre-incubated for 30 min at 37°C with pre-immune serum or FimA immunized serum diluted at 1:100 in serum free media. The number of bacteria for each well was 5 x 10^6 cells. The pre-incubated bacteria was added to the cells and incubated for 4 h. Bacterial suspension was removed and 1 ml of media containing 200 µg/ml metronidazole was added and incubated for a further hour to kill the bacteria outside the cells. Cells were washed twice with PBS and lysed by addition of 1 ml of distilled water and incubated for 30 min at room temperature. Cells were vortexed at maximum speed for 20 min as an extra step to disrupt the cells and release the bacteria. The lysates was then serially diluted and inoculated on ATCC medium 2722 supplemented Tryptic Soy (Difco, Sigma-Aldrich) agar plate. The plates were incubated in Forma anaerobic system (Thermo Scientific) at 37°C for 5-7 days. Number of bacteria inside H357 cells was reported as CFU/ml.

Statistical analysis

Unpaired t-test was used to analyze differences between groups with normal distribution. Mann-Whitney test with Welch's correction which does not assume equal standard deviation was used to analyze differences between groups those did not have normal distribution.

Results

FimA protein immunized rabbit produced anti-FimA and anti-*P.gingivalis* antibody

Two rabbits were immunized with 200 µg of purified FimA protein after pre-immune blood samples were collected. The rabbits were boosted on day 14, 28 and 42 with 100 µg FimA protein. Immunized blood samples were collected on day 49 for antibody check and further used for invasion assay. ELISA with purified FimA protein or formalin-fixed *P. gingivalis* showed that immunized rabbits produced IgG specific to both FimA and *P. gingivalis* (Fig. 1). The pre-immune sera contained very low level of anti-FimA or anti-*P. gingivalis* IgG. The antibody levels shown in Fig. 1 was only expressed the IgG levels when serum was diluted to 1: 800 or 0.00125 dilution. For FimA antigen, the same IgG level in immunized serum was maintained until the serum was diluted to 1:12800 and the absorbance at 490 nm was over 1.1 when the serum was diluted to 1: 51200.

Pre-immune serum from rabbit number 1 and number 2 (Pre 1 and Pre2) or FimA immunized sera from the 2 rabbits (Fim1 and Fim2) were collected for analyzed by ELISA using FimA protein or formalin-fixed P. gingivalis. The level of anti-FimA IgG (a.) or anti-P. gingivalis IgG (b.) was shown as the levels of absorbance at 490 nm.

Anti-FimA antiserum did not inhibit H357 invasion

Comparing to the group of *P. gingivalis* with serum or pre-incubated with pre-immune serum; number of *P. gingivalis* invading into H357 cells was higher when the bacteria were pre-incubated with serum from rabbit immunized with FimA protein (Fig. 2). Anti-FimA antiserum from one rabbit increased invading bacterial number significantly while another one seemed to increase the number as well but failed to reach significance. *P. gingivalis* that was not incubated with serum was also used in invasion assay. The number of invading bacteria in this group was low but the data could not compare statistically to the other groups since it was done only in duplicate. The invasion experiment was done 3 times independently and the similar results were observed.

P. gingivalis pre-incubated with pre-immune serum (Pre1 or Pre2) or FimA immunized serum (Fim1 or Fim2) or the bacterial suspension that was not incubated with any serum (NoSerum) was added to H357 cell culture and incubated for 4 h. Bacterial suspension was removed and the remaining bacteria outside the cells were killed with metronidazole. Cells were washed with PBS and

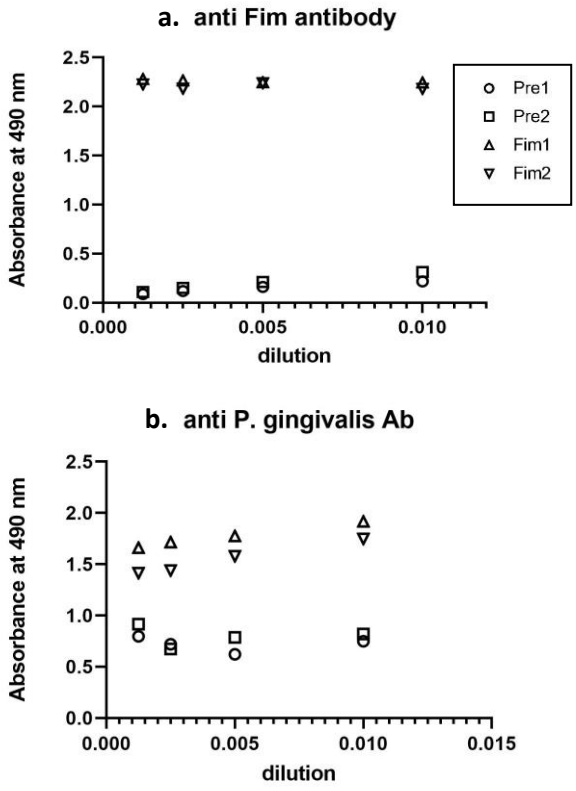

Figure 1. Antibody levels induced by FimA protein.

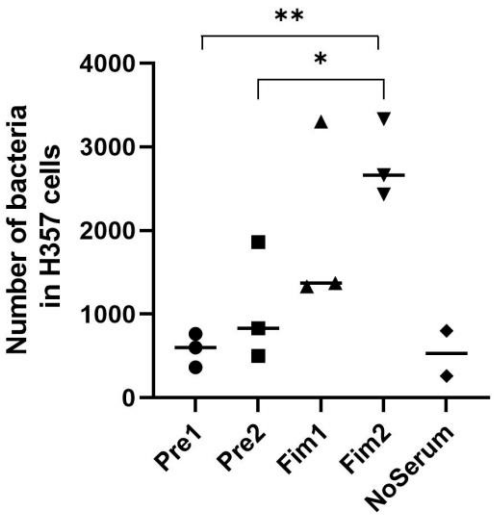

Figure 2. Effect of rabbit serum on *P.ginigvalis* invasion.

lysed by addition of distilled water and vortexing. The lysates was then serially diluted and inoculated on agar plate for quantification of bacteria inside H357 cells. Number of invading bacteria (Y axis) was shown in CFU/ml. *: $p<0.05$, **: $p<0.01$.

Discussion

Antibody production in rabbits can be induced by many methods. Some researchers or scientific companies immunize rabbit subcutaneously using antigen mixing with adjuvant such as Complete Freund's Adjuvant (CFA) or Incomplete Freund's Adjuvant (IFA).[12] In this study we chose intravenous route of antigen admistration which does not require adjuvant. The antibody titer was relatively high as at 1:51200 dilution we still could detect anti-FimA IgG with the absorbance at 490 nm over 1.1.

Incubation of anti-FimA antiserum to *P. gingivalis* before co-incubating the bacteria with H357 cells seemed to increase the invading bacteria number. This preliminary result was not what we expected at first, as many publications showed the inhibiton effect of anti-FimA antibody on *P. gingivalis* invasion.[7-9] This result will be needed to confirm using other techniques that can quantify number of invading bacteria. One of the techniques is to fluorescent label the live bacteria with before cell invasion assay. Number of invading bacteria can then be quantified under fluorescence microscope or flow cytometry.[13,14]

If the anti-FimA antibody can increase the number of *P. gingivalis* inside host cells, this might be through the function of IgG Fc receptor on host cell surface. However, the appearance of Ig Fc receptor on H357 cell surface has never been reported. There is also no report of Ig Fc receptor on normal oral epithelial cells, but there are evidences showing that FcRn is expressed in normal human epidermal keratinocytes and intestinal epithelial cells. Fc gamma receptors also express in human nasal epithelial cells, human melanoma and carcinoma cells.[15-19] Since rabbit IgG can bind to human Fc gamma receptor,[20] if H357 cells express Ig Fc receptor it is possible that anti-FimA IgG will enhance cell internalization of *P. gingivalis*.

Conclusion

Incubation of anti-FimA antiserum to P. gingivalis before co-incubating the bacteria with H357 cells increased the invading bacteria number.

Acknowledgements

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Evaluation of Different Scaffolds for Bone Regeneration in Rat Calvarial Bone Defects

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ABSTRACT

Background and Objective: Scaffold plays a key role in the context of tissue engineering. It not only provides a structural support for cells, but also creates an appropriate milieu for recruited cells, enhancing the therapeutic effects of cell-based treatments, and enabling the controlled release of biological cues such as growth factors. Collagen is widely used scaffold material in tissue engineering, particularly in periodontal and bone regeneration. Other natural polymers, however, have been developed and can be useful. Therefore, in this study, we aim to investigate the ability of four different natural polymers including collagen, chitosan, silk fibroin, and silk fibroin/gelatin hydrogel in promoting bone regeneration *in vivo* using rat calvarial bone defect.

Methods: Two critical-sized defects (5-mm diameter) were created on the right and left calvarium of 8-week-old male Wistar rats. The rats were randomly assigned to one of the four treatment groups (n=3-4/group) and implanted with collagen, chitosan, silk fibroin, or silk fibroin/gelatin hydrogel, respectively. Empty defect was used as a control. Four weeks after surgery, all animals were sacrificed and the calvarial bones were dissected for volume/total volume percentage (BV/TV%) measurement using micro-computed tomography, and subsequent histological analysis using hematoxylin & eosin staining.

Results: The collagen scaffold resulted in significantly higher BV/TV than the other groups ($p<0.05$) with the greatest amount of new bone formation. There was no significant difference between other scaffolds and control group.

Conclusion: Within the limitation of this study, collagen is the most effective scaffold in promoting bone regeneration in rat calvarial bone defects.

Keywords: Bone regeneration, Animal study, Collagen, Chitosan, Silk fibroin

Introduction

Periodontitis is a chronic inflammatory disease characterized by the destruction of tooth-supporting structures, particularly alveolar bone.[1] The goal of periodontal treatment is to control the inflammation and regenerate damaged tissues in an attempt to restore their normal functions and structures. The current outcome of guided tissue regeneration (GTR) and guided bone regeneration (GBR) is often unpredictable, in particular, when a complete regeneration is anticipated in severe cases.[2,3] Therefore, a concept of tissue engineering/regenerative medicine has been applied to periodontal regenerative therapy. The major components of tissue engineering-based approaches include cells, signaling molecules/growth factors, and scaffolds. These approaches can be used either alone or in combination with one another.[4]

Scaffolds are often required in bone regeneration to fill defects and provide an optimal milieu for cells infiltration and subsequent tissue formation.[5] In addition, scaffold can be used as a delivery platform for cells, bioactive molecules, and genes into the body.[6] As scaffolds for bone tissue engineering, polymers are widely used. Natural polymers such as collagen, gelatin, and chitin have several advantages, including high biodegradability, the presence of cell recognition and adhesion sites, similarity with native extracellular matrix, cytocompatibility, and low immunogenicity. Nevertheless they have some disadvantages, including poor mechanical properties and low stability.[7]

Collagen-based biomaterials have probably been the most widely used scaffold for bone tissue engineering and also other biomedical applications. Collagen is an essential component of the bone matrix, represents more than 90% of the organic mass of bone. Collagen presents as a template and may initiate and propagate the mineralization during bone formation process. Collagen scaffolds have good osteoconductive properties. However, no evidence of osteoinductive activity has been found in a non-osseous environment. Nonetheless, collagen-based biomaterials can be modified to include other bioactive domains to direct cells that are able to interact with the collagen towards certain differentiation pathways.[8]

Chitosan and its derivative are another group of natural biopolymers that have been explored for bone tissue engineering. Chitosan is a fully or partially deacetylated form of chitin, which can be found in fungi, exoskeleton of insects and shells of sea crustaceans. It contains positively charged amino acids, which contribute to its hemostatic and mucoadhesive capacity. Chemical structure of chitosan is similar to glycosaminoglycans (GAGs) which

are a major component of bone extracellular matrix. Their hydrophilic surfaces favorably facilitate cell adhesion, proliferation and differentiation. Additionally, chitosan is biodegradable, biocompatible, nontoxic, and has bacteriostatic properties.[9]

Silk fibroin is a natural polymer produced from many types of insects, especially from mulberry silkworm, *Bombyx mori L*, which mainly comprises of proteins, minor lipids, and polysaccharides.[10] Besides its biocompatibility and high oxygen permeability,[11] it has been shown to promote an alkaline phosphatase activation and collagen synthesis. Because of its ability to differentiate, secrete extracellular matrix, and mineralize, silk fibroin scaffold is advantageous as a tissue engineering scaffold on mesenchymal stem cells (MSCs).[10] Its bioinertness, which delays cell adhesion and limits cell growth, as well as its high water solubility and poor physiological stability, remain its main drawbacks.[12]

Previous studies have suggested combining silk fibroin with other materials, such as gelatin, to improve its properties. Gelatin is a collagen-derived natural polymer formed through hydrolysis of acid and alkaline. Gelatin has been used as a carrier in tissue engineering to deliver active molecules and cells. The active molecule of gelatin is arginine-glycine-aspartate (RGD) peptides which are cell adhesion factors that can enhance cell proliferation.[13] Silk fibroin/gelatin was developed and fabricated in a hydrogel form. A hydrogel is a three-dimensional network formed by the physical and chemical cross-linking of hydrophilic polymers via covalent bonds or physical intramolecular and intermolecular interactions. Without dissolving in water, hydrogel absorbs water and becomes inflated with soft and rubbery characteristics comparable to living tissue. In tissue engineering, hydrogel has been used as scaffolds due to its properties that resembles extracellular matrix, providing structural integrity, housing and delivering cells, and serving as tissue barriers and bioadhesives. In addition, hydrogel has the ability to absorb and deliver drugs and other bioactive molecules to enhance regeneration process.[14]

Therefore, in this study, we aim to investigate the ability of four different natural polymers including collagen, chitosan, silk fibroin, and silk fibroin/gelatin hydrogel in promoting bone regeneration *in vivo* using rat calvarial bone defect.

Materials and Methods

Animal

All rat experimental protocols in this study were reviewed and approved by Institute of Animal Care and Use Committee of Faculty of Tropical Medicine, Mahidol University (protocol number 006-2020). Wild-type Wistar male rats, aged 7-week old were purchased from Nomura Siam International Co., Ltd. (Bangkok, Thailand) and adopted in individually ventilated cages with 12-hours light/dark cycle for a week before beginning of the experiment. The rats were randomly divided into 5 groups of 3 to 4 rat each. The number of rats per group was determined with G power software, based on Elangovan, 2015.[15]

Preparation of scaffold

The collagen (CollaTape®) was purchased from Zimmer Dental (Carlsbad, CA, USA). The 5% w/v freeze-dried chitosan (85% degree of deacetylation) was fabricated at Petroleum and Petrochemical Collage, Chulalongkorn University, Thailand.[16] The 6.5 wt.% freeze-dried silk fibroin and silk fibroin/gelatin (30:70) hydrogel were fabricated at Faculty of Engineering, Chulalongkorn University, Thailand.[17]

In vivo implantation of scaffold

Animals were housed and cared in Mahidol university. Before starting the experiment, they rested for one week. Preoperatively, the 8 weeks old animals were anesthetized intraperitoneally (IP) by Zoletil (Virbac®) (40 mg/kg) and Xylazine (X-Lazine®) (5 mg/kg). They were disinfected with 2% Chlorhexidine and 70% Ethanol, followed by shaving from the bridge of the snout between the eyes to the caudal end of the skull/calvarium using electric clippers. Local anesthetic injection was performed with 2% Lidocaine with 1:100,000 Epinephrine (0.2 ml). Their eyes were protected with eye ointment. Sagittal incision in the scalp was made from the nasal bone to middle sagittal crest or bregma. Reflect full thickness flap was performed by using blunt instrument to separate bone from the underlying dura. Bone was excised and defects were created by generated two critical-sized defects on the right and left from midline (5-mm diameter, through to through) using trephine bur under continuously sterile saline irrigation. Group one received no treatment and, therefore, was used as a control. Group two to five were implanted bilaterally with collagen (CollaTape®, Zimmer Dental, Carlsbad, CA, USA), chitosan, silk fibroin, or silk fibroin/gelatin hydrogel. After scaffold implantation, the periosteum and scalp were closed from one layer at a time with interrupted 5-0 Vicryl resorbable sutures. Additional, Carprofen (Rimadyl®) (2.2 mg/kg) was injected to have an analgesic effect in all animals. They were sacrificed at 4 weeks after surgery and the calvarial bone was dissected for further analysis.

Micro-computed tomography (micro-CT) analysis

The dissected calvarial bones were fixed in 10% buffered formalin for 48 hours, before being scanned with X-ray micro-CT apparatus (SCANCO Medical AG, µCT 35, Switzerland). Scanned CT images were processed to quantify bone volume per total volume within the defect. 3D images were constructed using instrumentation software, a cylindrical region of interest (ROI), and a 3.5-diameter cylinder of sufficient height to cover the entire thickness of the calvarial bone.

Histological analysis

Following micro-CT analysis, calvarial bones were fixed in 10% buffered formalin for 24 hr and demineralized in 10% ethylenediaminetetraacetic acid (EDTA) solution. Paraffin blocks were sectioned with 5 µm thickness, and the sections were stained with H&E to analyze new bone formation and bridging of the created defects.

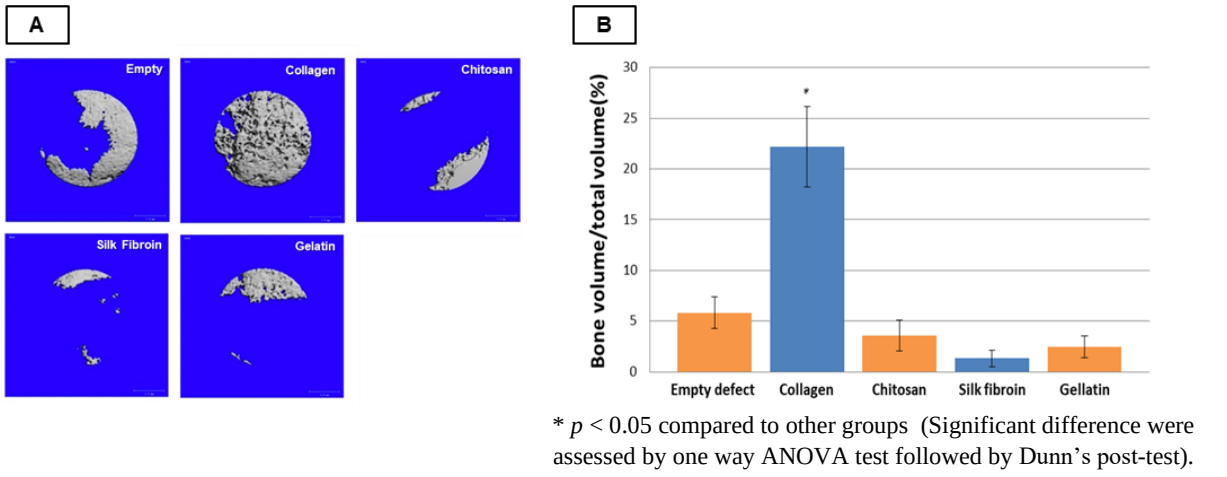
Statistical analysis

The volume/total volume percentage (BV/TV%) were analyzed using SPSS Statistics version 22.0 (SPSS Inc., Chicago, IL, USA). The normality distribution was determined by Shapiro-Wilk test. Parametric one way ANOVA test with Dunn's correction (followed by pairwise comparisons) were performed for multiple group comparisons, respectively. For all statistical analysis, *p*-value less than 0.05 were considered statistically significant.

Results

Micro-CT analysis was done to display bone regeneration in rat calvarial bone defects by analyzing the mineralized bone quantity and calculating the percentage of mineralized bone volume divided by the total tissue volume of interest (BV/TV%). The collagen group exhibited the most mineralized bone quantity among all groups at 4 weeks after scaffold implantation (Fig. 1A). The %BV/TV was also significantly higher in the collagen group ($p<0.05$) when compared with other groups, while no significant difference were found among other scaffolds and empty defects (Fig. 1B).

The results from micro-CT were confirmed by histological analysis. The new bone was significantly formed in the defects implanted with collagen scaffold. The defect bridging was also observed. Other groups showed only a small amount of new bone formation, with no significant difference from empty defects. Of note, high inflammatory cell infiltration was found in defects



* $p < 0.05$ compared to other groups (Significant difference were assessed by one way ANOVA test followed by Dunn's post-test).

Figure 1. (A) Representative µCT scans showing the level of regenerated bone after 4 weeks in empty defects (control group) and defects implanted with collagen, chitosan, silk fibroin and silk fibroin/gelatin hydrogel. (B) Bone volume fraction (BV/TV%) of regenerated bone after 4 weeks of implantation with different scaffolds (Data are presented as mean ± SD, n = 3-4).

implanted with silk fibroin and silk fibroin/gelatin hydrogel with substantial residual scaffold in the latter groups (Fig. 2).

Discussion

There is a need in the field of regenerative medicine for the development of novel scaffolding biomaterials, particularly for bone regeneration. Scaffold design is critical in bone regeneration/bone tissue engineering. In an intial step of physiologic bone healing, inflammatory phase, angiogenesis occurs along with the infiltration of various cell types and cytokines engaged in bone regeneration. Bone formation phase begins when fibroblasts, chondrocytes and osteoblasts take over. Granulation tissues formed in an inflammatory phase are gradually replaced by soft and hard callus formation. During the bone remodeling phase, hard callus remodels into the lamellar bone and the cancellous bone trabeculae through an orderly bone resorption and formation process mediated by a complex network of cells and cytokines. However, bone cannot heal properly when the damaged bone tissues are quite extensive. The use of scaffolds has become a potential solution to this challenge. Scaffolds provide a platform for blood clot stabilization and cell recruitment in the early stage of healing; thus, a biomimetic porous structure of scaffold is important. Mechanical strength and degradation process must be considered.[18] Scaffolds should survive environmental stress to act as a template during bone formation, and then degraded during bone remodeling phase. Many scaffolds have been introduced and studied such as polymers, ceramics, metal and composite.[19] None of these, however, are referred to as gold standards. Among different materials, collagen scaffold is one of the most widely used and commercially available for clinical application in various forms including sponge, membrane, powders/particles, fibers and gels.[8]

In this study, non-cross-linked type I collagen was compared with chitosan, silk fibroin and silk fibroin/gelatin in hydrogel. It was showed that collagen resulted in greatest new bone formation in rat calvarial bone defects. Our findings were in accordance with Song et al. 2007[20] that compared the same type of collagen (CollaTape®) in rat calvarial defects to empty defects. In their study, collagen scaffold was implanted for 8 weeks and showed more defect closure with

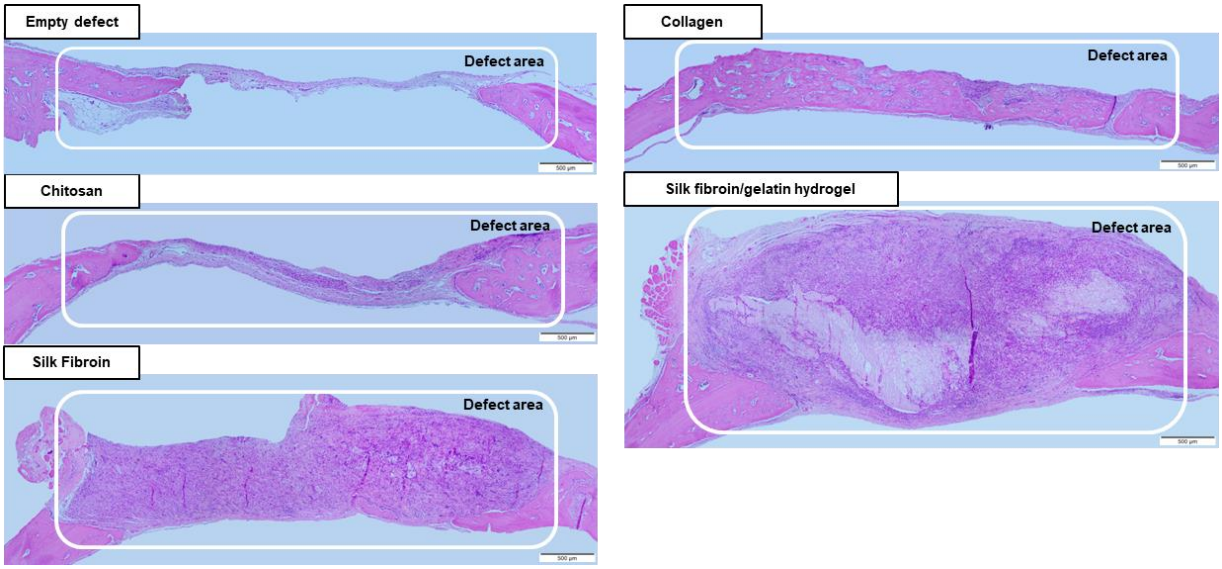


Figure 2. Illustrative histologic sections (4x) demonstrating the extent of new bone formation in the empty defect (control group) and defects implanted with collagen, chitosan, silk fibroin and silk fibroin/gelatin hydrogel.

greater bone formation than empty defects. However, Elangovan et al. 2015[15] found that collagen matrices implanted in rat calvarial defects resulted in little or no bone formation similar to empty defects after 4 weeks. This finding was similar to another study that tested collagen sponge in rat femoral defects. There were negligible bone volume/total volume% and bone formation in both empty and collagen sponge groups at 8 weeks after implantation.[21] The discrepancies in the ability of collagen scaffold to induce bone regeneration in rat experiment could be attributed to the heterogeneity of the animal used. Different strains and age were showed to have different osteogenic potential.[22,23]

Chitosan has been also investigated for its potential in inducing bone regeneration in many studies both *in vitro* and *in vivo*. The results were inconsistent.[16,24] Despite its attractive properties for tissue engineering, chitosan has limited water solubility, which is a significant disadvantage. To facilitate chitosan solubility, acid and/or chemical reagents must be used as solvents, which may result in toxic byproducts.[16] Furthermore, pure chitosan may have inadequate mechanical strength and high degradation rate, especially in acidic environments or in the human body where lysozymes are present. In our study, chitosan alone was not able to induce bone formation. However, chitosan might be used in combination with other growth factors.[24]

Silk fibroin has gained its interest as a potential material for bone scaffolding. Pure silk fibroin was investigated in rat calvarial defects and significantly induced new bone formation as compared to empty defects. The ability of silk fibroin in regenerating bone *in vivo* was enhanced when combine with betatricalciumphosphate (β-TCP).[25] This could be owing to its low stability and bioinertness,[12] which can be improved by incorporating with other molecules.[25] Silk fibroin cross-linked with gelatin demonstrated increased stability and cell adhesion *in vitro*.[12] However, silk fibroin/gelatin has never been tested *in vivo*. In our study, silk fibroin/gelatin hydrogel did not induce bone regeneration. The remnants of hydrogel were also found at the defect area. Other formulations of silk fibroin, such as silk fibroin/bioactive glass, silk fibroin/ gelatin/hyaluronan scaffold should be investigated in the future.[12,26]

Scaffolds used in this study have various pore diameters and resorption rates. Therefore, the results should be interpreted with caution. Rather than type of scaffolds, their overall physical and chemical properties should not be overlooked. Pore sizes and resorption rates, for example, may have an impact on bone regeneration. Although macropores (pore size larger than 50 µm) has advantage for osteogenic quality and cell infiltration, micropores (pore size less than 10 µm) provide greater surface area that leads to better ion exchange and bone protein absorption.[27] At present, the optimal pore size of bone tissue engineering scaffolds is still inconclusive, because different experimental settings (e.g., scaffold materials and bone defect site) often yield different results.[18] In our study, collagen has the smallest pore size, while silk fibroin has the largest pore size. Previous studies have demonstrated that collagen sponge (CollaTape®) resorbed quickly within 10 to 14 days.[28] Chitosan completely degraded in the sixth weeks *in vivo*.[16] While silk fibroin rapidly degraded and had just 19.87 percent remaining weight after immersion in de-ionized water for 24 hours,[26] silk fibroin/gelatin hydrogel had almost 100 percent remaining weight even after 10 days *in vitro*.[12] The scaffolds should have proper resorption rate that matches to the replacement of new bone growth,[19] which is approximately 2 to 3 weeks in rodents and 6 to 12 week in human.[29,30] According to our findings, a significant amount of silk fibroin/gelatin hydrogel was identified histologically 4 weeks after implantation. In comparison to silk fibroin and silk fibroin/gelatin hydrogel, collagen and chitosan may have an appropriate proper resorption rate. However, chitosan may contain toxic byproduct that interferes with bone regeneration. Another limitation of this study is the small sample size. Thus, larger sample sizes should be employed in future studies.

Conclusion

Within the limitation of this study, collagen is the most effective scaffold in promoting bone regeneration in rat calvarial bone defects after implantation for 4 weeks.

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Anti-Inflammatory Effect of *Gynura Procumbens* Extracts on *Aggregatibacter Actinomycetemcomitans* LPS-Stimulated Macrophage Cells

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ABSTRACT

Background and Objective: *Gynura procumbens* (GP) is a small plant found in Southeast Asia, China, and Africa. Several studies reported that *Gynura procumbens* Extract solution (GPE) has anti-inflammatory, antihypertensive, anticancer, antihyperglycemic, and anti-oxidative properties. Periodontitis is a bacterial-induced host-responsive inflammatory disease that relates to several systemic diseases. This study aimed to investigate the effect of GPE on tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) mRNA expression induced by lipopolysaccharide (LPS) of a periodontal pathogen in macrophages.

Methods: The GP leaves were prepared by the aqueous extraction technique. Mice macrophages RAW 264.7 cells were seeded overnight before being stimulated by the combination of LPS of *Aggregatibacter actinomycetemcomitans* (*A.a.*) and GPE (25, 50, 100, 250, 500 and 1,000 µg/mL) for three hours. Cells were collected and analyzed mRNA expression of TNF-α and IL-1β by quantitative RT-PCR. The toxicity of GPE to cells was examined by Cell Counting Kit-8 (WST-8) assay.

Results: The WST-8 result showed that GPE concentrations between 10 - 250 µg/mL were biocompatible with RAW 264.7 cells. Although GPE alone does not affect a remarkable change in TNF-α and IL-1β levels, we found that GPE (100 and 250 µg/mL) significantly suppressed TNF-α and IL-1β mRNA expression induced by *A.a.* LPS.

Conclusion: The GPE 100 and 250 µg/mL presented anti-inflammatory effects on significantly reduced TNF-α and IL-1β mRNA levels activated by LPS of *A.a.* with no toxicity to mice macrophage cells.

Keywords: *Gynura procumbens*, IL-1β, periodontitis, TNF-α

Introduction

Gynura procumbens (GP) is a medicinal plant commonly found in China and Southeast Asia, such as Thailand, Indonesia, and Malaysia,[1] and is traditionally used to treat several health problems, including inflammation, fever, rashes, hypertension, diabetes mellitus, and cancer.[2] In addition, GP leaves were also used for cooking and were safe to eat raw. The effects of *Gynura procumbens* extract solution (GPE) on various diseases were reported in several scientific studies, such as anti-inflammation,[2-7] antioxidant,[2-3,8] antihypertension,[2,9] antihyperglycemia,[2,10] cholesterol level reduction,[11] anticancer,[2,12] antibacterial,[2,6] and antiviral.[13] From the above properties, many studies pointed to the flavonoids and phenolic compounds, which were determined as the active compounds of GP.[4,6,8,14] Flavonoids are the secondary metabolites of plants usually found in leaves, seeds, bark, and flower. The important properties of flavonoids are their antioxidant, anti-inflammatory, and anticancer effects.[15] Phenolic compounds also have the same properties as flavonoids.[16] Since the anti-inflammatory property of GPE was widely investigated, it is clear that the GPE suppresses the inflammation via the PI3k/Akt and MAPK signaling pathway, inhibits Nitric oxide (NO) and cyclooxygenase-2 (COX-2) production,[5] and reduces the production and secretion of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), Interleukin-1 beta (IL-1β).[3,5,17] Previous studies reported that GPE could alleviate inflammation comparable to 6 mg of Hydrocortisone[4] and stimulate some anti-inflammatory cytokine secretion, such as IL-10.[7] As the properties mentioned above, GPE may have the potential to be developed as an alternative treatment or a new product for curing inflammatory-related diseases.

Periodontitis is one of the inflammatory-related diseases located at the oral periodontium, resulting in the destruction of the periodontium and alveolar bone.[18] *Aggregatibacter actinomycetemcomitans (A.a.)* is one of the significant periodontal pathogens of periodontal disease and is frequently found in severe periodontitis patients.[19] Lipopolysaccharide (LPS), the virulence factor of gram-negative bacteria, is also found in *A.a.* and activates host immune response through the Toll-like receptor 4 (TLR4) on white blood cells such as polymorphonuclear neutrophils (PMNs) and macrophages to produce cytokines for bacterial elimination. The major proinflammatory cytokines, such as TNF-α and IL-1β, play roles in inflammation response in the oral cavity. High TNF-α and IL-1β levels may lead to more severe inflammation and express more destructive periodontium.[20] The initial phase treatment of periodontal disease by scaling and root planning is the gold standard for controlling inflammation. However, bacteria are hard to completely eliminate in deep pockets because of the limitation of instrument access. *A.a.* is one of the periodontal pathogens with a remarkable ability to avoid host immunity invading gingival tissue.[19]

Therefore, new innovations and techniques to control periodontal inflammation are challenging to improve humanity's quality of life. Moreover, a study about the anti-inflammatory effect of GPE in periodontal disease was not found. This study aimed to investigate the anti-inflammatory property of the GPE on the *A.a.* LPS-stimulated macrophage cells. The cytotoxicity test was also performed to confirm the biocompatibility of GPE in mice macrophage cells.

Materials and Methods

Plant materials

The GP leaves were purchased from a local supplier in Bangkok, Thailand, in November 2021. The leaves were cleaned with deionized water and air-dried at room temperature. Then, GP leaves were further dried in a hot air oven (Memmert UM 400, Memmert GmbH & Co. KG, Schwabach, Germany) at 40 °C for three days and were finally ground into powder by an electric blender.

Gynura procumbens leaves extraction and preparation

GP leaves extracts were prepared based on the maceration method.[7] The 200 g of GP leaves powder was submerged in 1.5 L of deionized water. The maceration process was conducted in a temperature-controlled water bath (Eyela OSB-2000, Tokyo Rikakikai Co. Ltd, Tokyo, Japan) at 50 °C. The mixture was intermittently stirred every 20 minutes for 3 hours. Then, the obtained solution was filtered by a vacuum filtration apparatus with Whatman No. 1 filter. Finally, the filtered solution was concentrated by a vacuum freeze dryer (Faithful FSF-12N, Hebei, China) and stored at 4°C in a desiccator. Dried GP extract was dissolved in deionized water to 10 mg/mL concentration as a stock solution for this study.

Cell culture

RAW 264.7 macrophage cells were provided by American Type Culture Collection (ATCC). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Grand Island, NY, USA) with 10% Fetal Bovine Serum and 1% penicillin-streptomycin (Gibco, Grand Island, NY, USA). The cells were incubated in a CO_2 incubator with a 5% CO_2 atmosphere at 37 °C and were subcultured when the cells reached 80% confluent or every three days.

Cytotoxicity test

The cytotoxicity test was evaluated to determine the appropriate concentration of GPE that is not harmful to macrophage cells. In this study, we examined the cytotoxic of GPE by Cell Counting Kit-8 assay (Abcam, Cambridge, UK) and calculated the percentage of cell viability of the test groups compared to the control group. WST-8 solution was used to test the cellular function. Living cells would change WST-8 tetrazolium salt to orange formazan products. RAW 264.7 cells were seeded in a 96-well plate (5 x 10^5 cells/well). The various concentrations of GPE (10, 25, 50, 100, 250, 500, and 1,000 µg/mL) were added into each well except for the control group and incubated for 48 hours. After that, the WST-8 solution was added to each well and additionally incubated for 2 hours in the dark. Measurement of the absorbance was processed by the microplate reader (MULTISKAN sky, Thermo Fisher Scientific, Massachusetts, USA) at the wavelength of 460 nm.

The analysis of TNF-α and IL-1β mRNA expression by Quantitative RT-PCR

RAW 264.7 (1 x 10^6 cells) were seeded in a 6-well plate and cultured in DMEM with 5% FBS overnight. The *A.a.* LPS (1 µg/mL) was added with or without GPE (25-1,000 µg/mL). Then cells were incubated for 3 hours and collected for real-time PCR assessment. The total RNA was extracted by using the RNeasy® Mini kit (Qiagen, Maryland, USA) and converted to cDNA by ReverTra Ace™ qPCR RT Master Mix (Toyobo Co, Ltd, Osaka, Japan). In this study, the mRNA expression of TNF-α and IL-1β was performed by quantitative RT-PCR technique. The sequences for forward and reverse primers of TNF-α, IL-1β, and GAPDH, which was considered as an internal reference, were shown in Table 1. Reaction volumes consisted of LightCycler 480 SyBr Green I Master (Roche Applied Science, Rotkreuz, Switzerland), mixed primers, and cDNA prepared before. The RT-qPCR was processed by LightCycler 480® (Roche Applied Science, Rotkreuz, Switzerland), pre-incubation at 95°C for 30 seconds, followed by 40 cycles of denaturation at 95°C for 5 seconds, and annealing at 58°C for 40 seconds, extension at 72°C for 45 seconds, and finally cooling at 40°C for 30 seconds. The relative expression of each gene was calculated by the LightCycler 480 program according to the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

The data were analyzed by SPSS statistics 26.0 (IBM, Armonk, NY, USA) and expressed as mean ± standard deviation. Analyzing the comparison between the control group and test groups was performed by independent *t*-test and the comparison between multiple groups was performed by one-way ANOVA followed by Tukey's test. A *p*-value < 0.05 was considered statistically significant.

Table 1. List of primers sequence.

Genes		Primer Sequence (5' – 3')
TNF-α[21]	Forward primer	CATCTTCTCAAAATTCGAGTGACAA
	Reverse primer	TGGGAGTAGACAAGGTACAACCC
IL-1β[22]	Forward primer	AAGCTCTCCACCTCAATGGACAG
	Reverse primer	CTCAAACTCCACTTTGCTCTTGA
GAPDH[23]	Forward primer	GAGAAACCTGCCAAGTATGATGAC
	Reverse primer	TAGCCGTATTCATTGTCATACCAG

Results

Effects of GPE on cell viability

CCK-8 assay was performed to examine the cytotoxicity of GPE in mice macrophage cells. Each test group's cell viability percentage was compared to the control group, which was considered 100% viability. The results (Fig. 1) demonstrated that GPE at concentrations of 10-250 µg/mL had more than 90% cell viability and no significant toxicity compared to the control group. However, the GPE concentrations of 500 and 1,000 µg/mL showed diminished proliferation of RAW 264.7 cells, which could be implied that the GPE concentration higher than 500 µg/mL had a toxicity effect on mice macrophage cells.

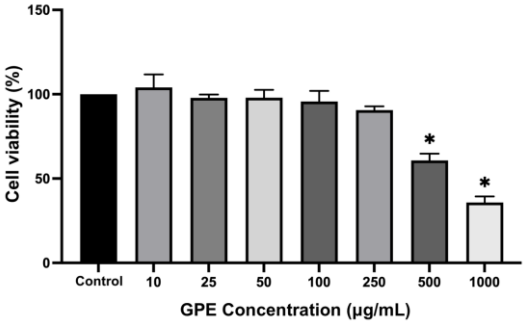

Figure 1. The percentage of viable cells depended on various GPE concentrations examined by CCK-8 assay. Data were expressed as mean ± SD (n = 3). * *p*-value < 0.05 compared with respective control using independent *t*-test.

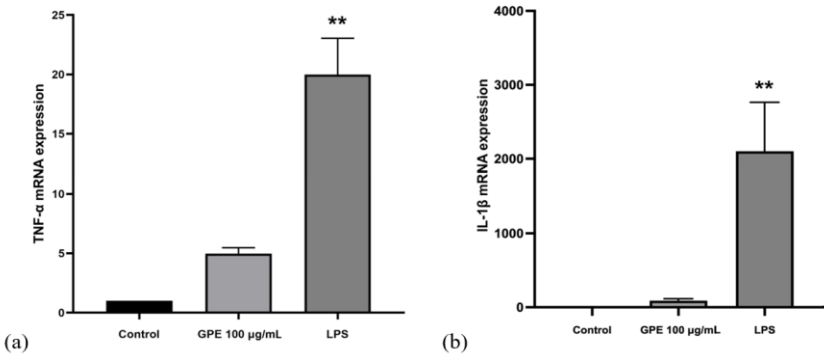

Figure 2. The effects of GPE on TNF-α and IL-1β expression in RAW264.7 cells assessed by RT-qPCR technique. Data were presented as mean ± SD (n = 3). ** *p*-value < 0.01 as compared with the LPS group. (a) TNF-α mRNA expression. (b) IL-1β mRNA expression.

Effects of GPE on macrophage cells

Effects of GPE on mRNA expression levels of TNF-α and IL-1β were analyzed by quantitative RT-PCR. TNF-α and IL-1β mRNA expression levels of the LPS-treated group exhibited a remarkable increase compared with the control group. Conversely, the GPE group did not show a significant change. TNF-α and IL-1β mRNA levels of the GPE group slightly elevated from the control group without statistical significance (Fig. 2).

Effects of GPE on LPS-induced macrophage cells

To clarify the anti-inflammatory effect of GPE, the various concentration of GPE were prepared and added to LPS-activated macrophages. RT-PCR results showed significantly elevated TNF-α mRNA expression levels of the LPS-stimulating RAW 264.7 group. However, TNF-α mRNA expression levels were significantly suppressed by GPE concentrations of 50, 100, and 250 µg/mL (Fig. 3). Similarly, IL-1β mRNA expression levels significantly reduced the augmentation of LPS-stimulated RAW 264.7 cells (Fig. 4).

Discussion

Gynura procumbens (GP) is a plant abundantly found in Thailand and known as an evergreen plant that can easily cultivate. GP has been widely used in folk medicine with copious scientific evidence of GP properties, including anti-inflammation,[2-7] antioxidation,[2-3,8] antihyperglycemia,[2,10] anticancer,[2,12] and antibacterial.[2,6] In this study, the anti-inflammatory effect of GPE on *A.a.* LPS-stimulated murine macrophage cells was assessed by examining TNF-α and IL-1β. Although there are various proinflammatory cytokines, such as IL-6, PGE_2, and MMPs, the evidence reported that TNF-α and IL-1β are crucial proinflammatory cytokines. These cytokines could enhance the synthesis and secretion of other mediators, including PGE_2, IL-6, and Nitric oxide

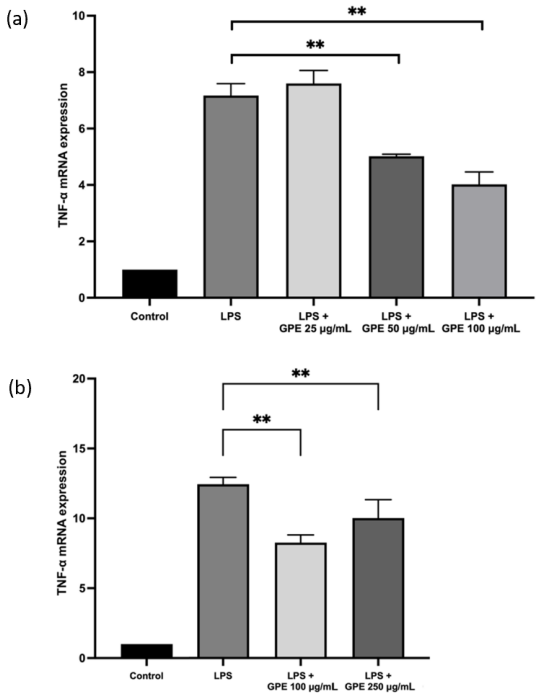

Figure 3. The effects of GPE on TNF-α mRNA expression in LPS-stimulated cells. (a) concentrations of 25, 50, and 100 µg/mL (b) concentrations of 100 and 250 µg/mL. Data were shown as mean ± SD (n = 3). ** *p*-value < 0.01 as compared with the LPS group.

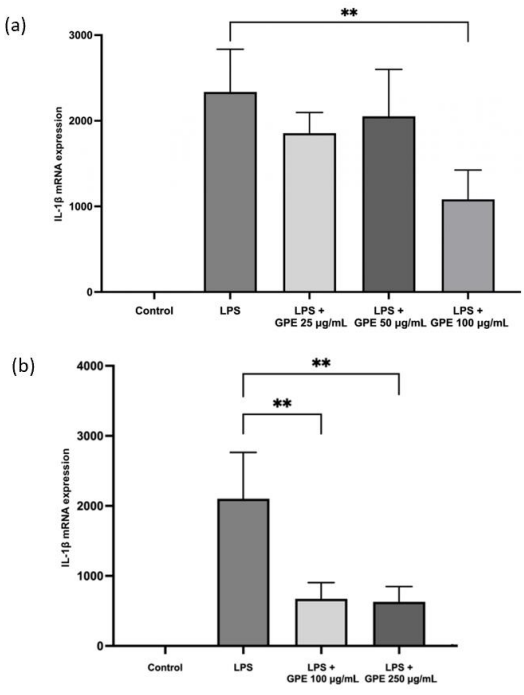

Figure 4. The effects of GPE on IL-1β mRNA expression in LPS-stimulated cells. (a) concentrations of 25, 50, and 100 µg/mL (b) concentrations of 100 and 250 µg/mL. Data were shown as mean ± SD (n = 3). ** *p*-value < 0.01 as compared with the LPS group.

(NO). The effect of GPE in decreasing TNF-α and IL-1β mRNA expression suggests that this extract could suppress inflammation.

The previous studies attempted to determine the phytochemical compositions of the GPE, which may have flavonoids and phenolic compounds are the primary active components, including chlorogenic acid, rutin, astragalin, and quercetin,[8,14] and each component also had the characteristic of flavonoids and phenolic compounds group. Although each purified component had its anti-inflammatory effect, the crude extracts of GP expressed a more anti-inflammatory effect.[6] This is probably because there are several active components in the crude GP extracts and these components work together. Therefore, the crude GP extract was selected in this study. Besides, considering the solvent used in the extraction process, GPE in this study was aqueous extracted, which was different from other studies. Further study of the phytochemical ingredients should be focused on.

Since this study was interested in the effect of GPE on periodontitis, an infectious disease characterized by chronic inflammation, The inflammation was simulated by using the *A.a.* LPS, which is one of the periodontal pathogen's virulence factors. LPS was regularly used in many *in vitro* inflammatory model studies. In this study, 1 µg/mL of *A.a.* LPS was used to induce the macrophage cells. The results showed a significant increase in TNF-α and IL-1β compared to the LPS group with the control group. When the various concentrations of GPE were added, the mRNA expression of TNF-α and IL-1β seemed to decrease. Corresponding to the previous study, GPE showed suppressive inflammation properties. In this study, the optimum concentrations of 100 and 250 µg/mL GPE showed satisfactory results in significantly reducing the levels of TNF-α and IL-1β with no toxicity to RAW 264.7 cells. Additionally, the GPE concentration of more than 500 µg/mL did not diminish the inflammation and was also toxic to RAW 264.7 cells. Conversely, some studies suggested that the anti-inflammatory effect of GPE was dose-dependent.[6,24] In future research, the enzyme-linked immunoassay (ELISA) may be performed to confirm the production of TNF-α and IL-1β proteins and the effect of GPE may be clarified at the molecular biological level.

The murine macrophage model was commonly used in the study of traditional medicine to treat inflammation because macrophage cells are essential cells in the inflammatory process.[25] From our results, the GPE may potentially reduce the inflammation related to periodontitis, which is the voluntary primary data for further clinical application for periodontal treatment. However, the first barriers of the periodontal pathogens were gingiva and PDL, the study effect of GPE in gingival fibroblasts and PDL cells needed to clarify in further studies.

Conclusion

In summary, the GPE concentration of 100 and 250 µg/mL has an optimal anti-inflammatory effect on the *A.a.* LPS-stimulated macrophage cells by reducing the TNF-α and IL-1β mRNA expression and had no toxicity to cells. Future investigations should focus on the impact of GPE on human cells and the clinical application of this extract.

Acknowledgements

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OM-00025

Saliva Profile of Patients with Primary Burning Mouth Syndrome Using ATR-FTIR Spectroscopy

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ABSTRACT

Background and Objective: Changes in saliva composition of patients with primary burning mouth syndrome (BMS) have been linked to its symptomatology. Fourier transform infrared (FTIR) spectroscopy is a non-invasive method that can characterize saliva, creating a novel platform for evaluating disease etiology and pathogenesis. This study aims to identify the specific biochemical spectral signatures of saliva in patients with primary BMS using ATR-FTIR spectroscopy.

Methods: Unstimulated whole saliva was collected from 10 patients with primary BMS and 10 healthy controls (HC). Saliva supernatant was extracted by centrifugation at 3,000x g for 20 minutes at 4 °C. The mid-IR absorbance spectra (4000 – 400 cm-1 range) were measured using the Tensor II, Bruker Spectrometer® equipped with attenuated total reflectance (ATR) attachment. Partial Least Squares (PLS) was utilized to build a discrimination model for the absorbance spectra of BMS and HC.

Results: A significant difference in absorbance intensity between the two groups in all bands was observed, with a higher absorbance intensity for most recorded spectral peaks in BMS. PLS of the normalized second derivative spectra showed discrimination between BMS and HC; 2-dimensional scores plot at factor 1 vs. factor 2 and factor 2 vs. factor 4 exhibited a distinct separation of the spectra between groups at 1800 – 900 cm^{-1} and 3000 – 2700 cm^{-1}, respectively.

Conclusion: The saliva of primary BMS patients can be discriminated from HC using ATR-FTIR spectrometry. An increased absorbance intensity for several salivary components is noted in BMS patients, which may help to understand the etiology and provide a more targeted approach for treating the disease.

Keywords: BMS, FTIR spectroscopy, Saliva

Introduction

Burning mouth syndrome (BMS) is a recurrent diurnal intraoral burning or dysaesthetic sensation persisting for more than two hours per day for more than three months without evident causative lesions on clinical examination and investigation.[1] It usually involves the tongue, palate, and gingiva, while a small number of patients report the involvement of the lips and buccal mucosa.[2] Several authors reported different estimated prevalence rates of 0.7 to 4.6%,[3] 1 to 3.7%,[4] and 0.11% with a higher incidence in elderly females.[5] Main symptoms of BMS comprise a triad involving pain in the oral mucosa, dysgeusia, and xerostomia. The burning sensation may be felt by patients in association with xerostomia and dysgeusia despite having normal salivary flow, with xerostomia being reported as a frequent symptom.[6]

Despite its complexity, saliva has been currently used as a diagnostic sample.[7] Advantages of using saliva include easier, safer, and more economical collection than blood while providing real-time diagnostic value.[8,9] Salivary diagnostics has a wide area of application from condition onset, diagnosis and monitoring, prognosis, and evaluation of therapeutic outcomes. It harbors the potential for biomarker identification using proteomic, genomic, and transcriptomic approaches for oral and systemic diseases.[10] Several researchers have analyzed the composition of saliva from patients with BMS using different methods such as sialochemistry. These methods were used to assess concentrations of proteins, calcium, potassium, chlorides, and others in saliva. Kang *et al*. performed a real-time PCR to quantify mucin 1 (MUC1) mRNA levels relative to the β-actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA levels and used enzyme immunoassays to analyze the cortisol, progesterone, 17 β-estradiol, and dehydroepiandrosterone (DHEA) concentrations from the saliva of BMS patients.[11] Other researchers used isobaric tags for relative and absolute quantitation labeling and liquid chromatography-tandem mass spectrometry to quantify salivary proteins.[12] A recent tool used for qualitative analysis of saliva, is Fourier transform infrared (FTIR) spectrometry, in which infrared spectra of the biomolecular composition of saliva from patients with BMS were recorded and compared with healthy saliva.[13]

FTIR spectroscopy is an analytical technique that uses infrared light in scanning samples to observe chemical properties by

identifying organic, polymeric, and inorganic materials.[14] It has been used for many biological specimens such as soft tissues, blood, urine, and saliva.[15] Rather than focusing on morphological changes in tissues, FTIR relies on the biochemical changes underlying the pathogenesis diseases offering high sensitivity, specificity, and accuracy of 81.3%, 95.7%, and 89.7%, respectively, as noted in studies involving differentiation of leukoplakia with oral squamous cell carcinoma.[16,17]

Utilization of FTIR spectroscopy in BMS patients to affirm changes in salivary composition at a molecular level showed increased nucleic acids and thiocyanate and decreased proteins and lipids compared to healthy controls.[13] Interestingly, FTIR can provide information about salivary components at a molecular level to correlate with symptoms and aid in disease monitoring. Currently, there is limited data regarding saliva analysis in primary BMS patients using ATR-FTIR spectroscopy. Thus, this study aims to analyze the specific spectral signatures of salivary biomolecules of primary BMS patients using ATR-FTIR spectroscopy. Primarily to determine if specific spectral signatures of saliva can discriminate between patients with primary BMS and healthy patients.

Materials and Methods

The study has been certified by the Human Research Ethics Committee of Khon Kaen University (HE652018) and was conducted under the Declaration of Helsinki and the guidelines provided by the International Committee on Harmonization of Good Clinical Practice (ICH-GCP).

Patients from the Oral Medicine and Orofacial Pain Clinic, Faculty of Dentistry, Khon Kaen University, with primary burning mouth syndrome following the diagnostic criteria of ICOP,[1] were recruited for the study. Generally, 20-80-year-old healthy patients, who are experiencing primary BMS for at least 3 months were included. Patients who smoke or have uncontrolled systemic conditions, oral lesions simulating burning sensation, or periodontitis were excluded from the study.

Likewise, healthy, sex-matched controls without BMS history, aged 20-80 years old, non-smokers, without oral lesions, and without periodontitis were recruited. Bleeding on probing was assessed for all patients and periodontal conditions limited to gingivitis or gingivitis on a reduced periodontium were included in the study. All patients must be able to read, write, and understand the information regarding the experiment. Informed consent was secured from all research participants.

Saliva Acquisition and Storage

Saliva collection was performed in the morning, from 9:00 to 11:00 AM. The spitting method was utilized for saliva collection. Patients were requested to be NPO and to brush their teeth at least an hour before saliva collection. The saliva was allowed to accumulate on the floor of the mouth then the patient spat directly onto a sterile conical centrifuge tube every 60 seconds for five minutes.[18] Collected saliva was refrigerated immediately at 4° C until further processing. In the laboratory, the saliva was vibrated for five minutes in the Vortex Genie 2®. The vortexed saliva was then transferred into 1.5 mL Eppendorf tubes and was centrifuged at 3,000x g for 20 minutes at 4° C. The saliva supernatant was separated and kept frozen at -80° C until ATR-FTIR analysis.

ATR-FTIR Spectroscopy

ATR-FTIR spectroscopy was performed at the Center for Innovation and Standard for Medical Technology and Physical Therapy, Faculty of Associated Medical Sciences, Khon Kaen University. The Tensor II, Bruker Spectrometer® equipped with attenuated total reflectance (ATR) attachment, was used for spectroscopy. In preparation for ATR-FTIR analysis, the frozen saliva supernatant was thawed at room temperature. 5 µL of saliva was dropped directly on the reflective surface of the FTIR spectrometer and air-dried with cool air for 10 minutes. Scans were performed in triplicate for each sample. The reflective surface of the machine was cleaned with deionized water and absolute methanol between each specimen scan.

IR Spectra Acquisition and Processing

IR spectra were obtained following the spectral resolution of 4 cm^{-1} with 64 co-added scans for both background and samples at 4000 – 400 cm^{-1} spectral range.

All spectra obtained from the saliva of all groups were analyzed using OPUS version 7.0 software and The Unscrambler®. Spectral scattering was first corrected with extended multiplicative signal correction (EMSC), then the second spectral derivative was obtained from the corrected spectra and smoothed using Savitzky-Golay derivative filter with 17 polynomial order to obtain good quality spectra for analysis. Two spectral regions, the fingerprint region (1800 – 900 cm^{-1}) and the C-H region (3000 – 2800 cm^{-1}) were selected for sample group classification.

Data Analysis

The mean of the raw spectra for both groups was plotted while dominant peaks in the spectra of each group were recorded. Descriptive statistics were used for each variable, and the Shapiro-Wilk test was performed to test for the normality of data. IBM SPSS® version 26.0 software was used for statistical evaluation in which $p<0.05$ was considered statistically significant.

Independent samples t-test was used to determine whether the mean difference of IR spectra was significantly different between the two groups. Partial least squares (PLS) was used on the spectra in the fingerprint (1800 – 900 cm^{-1}) and the C-H regions (3000 – 2800 cm^{-1}) to classify test spectra into their respective experimental classes. PLS data matrix consisted of an X variable, constituting the spectral data, and two Y variables, 1 and -1 integer values, coding for BMS and HC, respectively.

Results

Demographic and clinical characteristics

The demographic data of the patients is summarized in Table 1. A total of 10 patients were included in the BMS group, 9 female and 1 male ranging from 47 to 70 years old (mean±SD: 60.7±7.92). Five patients complained of burning pain and xerostomia only, while dysgeusia was a reported complaint together with burning pain and xerostomia for the other five patients. None of the patients reported a known trigger factor as symptoms were felt spontaneously throughout the day. Four patients reported gastroesophageal reflux disease (GERD) and gastritis, while one patient reported controlled hypertension (HTN) and dyslipidemia. The tongue was the most commonly reported location of the pain as reported by 90% of the patients, while 60% pointed to the buccal mucosa, and 40% reported pain on the lips. The mean unstimulated saliva flow rate (USFR) was 0.61±0.23 ml/min for the BMS group and 0.77±0.15 ml/min for the HC. The mean pH was 5.87±0.78 for the BMS group, while the HC had a mean pH of 7.08±0.13.

The HC consisted of 10 gender-matched, healthy subjects, aged 35 to 61 years old (mean±SD: 51.90±5.13), without any reported history of systemic disease or drug/medication use.

FTIR spectra of BMS and HC

The normalized mean spectral absorption is shown in Fig. 1. A significant difference in peak intensity between the two groups in all absorbance bands was observed. In the BMS group, the absorbance intensity of most recorded spectral peaks was higher than HC except 3273 cm^{-1}. Peak assignments indicated from the mean absorption bands are summarized in Table 2. The peak in 3273 cm^{-1} corresponds to stretching vibrations of NH Amide in nucleic acids. Both BMS and HC showed similar signature peaks in the fingerprint region with an

Table 1. Patient demographics and clinical data of the BMS group.

BURNING MOUTH SYNDROME								
	Age	Sex	General Health Condition	Drug/Medicament	Symptom/s	Trigger Factor	Location of Pain	Duration (months)
1	47	F	-	-	Burning pain, Xerostomia, Dysgeusia	None	Tongue	5 ½
2	56	M	GERD	-	Burning pain, Xerostomia, Dysgeusia	None	Tip of tongue, lower lip	6
3	70	F	GERD	-	Burning pain, Xerostomia	None	Lips, L&R buccal mucosa	24
4	66	F	Gastritis	-	Burning pain, Xerostomia	None	Tongue, L&R buccal mucosa	48
5	62	F	-	-	Burning pain, Xerostomia, Dysgeusia	None	Tongue, L&R buccal mucosa	60
6	69	F	GERD	Alendronate, Lyrica, Atorvastatin, Loxonin, Valdoxan	Burning pain, Xerostomia, Dysgeusia	None	Tongue, L&R buccal mucosa	7
7	64	F	-	-	Burning pain, Xerostomia	None	Tongue, L&R buccal mucosa, Lips	72
8	59	F	HTN, Dyslipidemia	Madiplot, Atorvastatin	Burning pain, Xerostomia, Dysgeusia	None	Tongue, L&R buccal mucosa, Lips	48
9	65	F	-	Aspirin	Burning pain, Xerostomia	None	Tongue	120
10	49	F	-	-	Burning pain, Xerostomia	None	Tongue	7

increased intensity pronounced in the bands of Amide I (1638 cm^{-1}) and Amide II (1544 cm^{-1}), corresponding to proteins. Increased absorbance intensity was also observed in the bending vibrations of CH_2 and CH_3 (1445 cm^{-1}), symmetric stretching vibrations of COO^- (1403 cm^{-1}), and asymmetric stretching vibrations of PO (1243 cm^{-1}), relating to lipidic and fatty acid changes. An increased absorbance intensity could also be noted in C – O – C stretch (1071 cm^{-1}) and stretching vibrations of C – C, C – O, and CH_2OH (926 cm^{-1}) related to sugars and glycosylated proteins. Additionally, bands at 2112 cm^{-1} and 2059 cm^{-1}, assigned to the symmetric stretching of thiocyanates have been recorded. Comparison of the mean absorbance at each dominant peak showed no statistical significance in most of the recorded peaks, except for the bands corresponding to 653 and 617 cm^{-1}, which showed a statistically significant difference between groups (p<0.05).

PLS of the average second derivative at the fingerprint and C-H regions was employed to discriminate the salivary infrared spectral data between BMS and HC. The 2-dimensional scores plot at factor 2 vs. factor 4 of the C-H region and at factor 1 and factor 2 of the fingerprint region are represented in Fig. 2A and 2C. The samples were clustered distinctly, with the BMS separated from the HC, especially in the fingerprint region. The loadings plot shows a marked difference in absorbance bands, as observed in Fig. 2B and 2D. From this result, the BMS and HC can be discriminated by proteins, lipids, and nucleic acid represented in the fingerprint spectral region.

Discussion

The present study demonstrated the potential of FTIR analysis for identifying the biochemical spectral signatures of saliva in patients with BMS and its potential for discrimination between healthy and diseased saliva. The study involved 10 patients who exhibited either 2 or 3 of the triad of symptoms associated with BMS.[1] All patients complained of subjective xerostomia but presented with a mean USFR of 0.61±0.23 (ml/min), which does not indicate hyposalivation; therefore it is difficult to trace whether the subjective feeling of dryness resulted from sensory changes brought about by burning pain or by hyposalivation.[6] However, all BMS patients presented with a decreased pH with a mean of 5.87±0.78 for the BMS group, which was significantly lower than the HC, which had a mean pH of 7.08±0.13 (p<0.05). This is in accordance with a previous study which showed a mean pH of 5.79±0.4 for BMS patients; nonetheless, that report did not describe the exact relationship of a decreased salivary pH with burning sensation.[21]

The human saliva has a rich composition of biomolecules as well as bacteria and their metabolic products, which provides a real-time diagnostic value.[8,9] The ATR-FTIR analysis of saliva in the present study showed several dominant peaks related to proteins, lipids, and nucleic acids in the BMS group. This result is contrary to the findings of Rodrigues *et al.*, which demonstrated a decreased absorbance intensity of proteins and lipids for BMS.[13] The increased absorbance

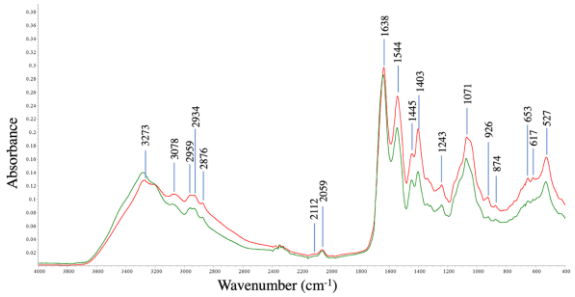

Figure 1. Mean absorption bands of the BMS (red) and the HC groups (green), indicative of peaks and intensity of absorbance of the major functional groups of biomolecules from the saliva.

of proteins in the present study might be related to an increase in salivary α-amylase, an enzyme containing dominant protein chains, as it was found to have increased levels in BMS. An increased α-amylase was reported to be associated to the stress and pain experienced by patients.[22] Furthermore, in a biomarker evaluation experiment in 2017, it was noted that a total of 39 proteins showed significantly increased levels in BMS patients, with a particular interest in alpha-enolase, interleukin-18 (IL-18), kallikrein-13 (KLK-13), and cathepsin G; reporting that overexpression of alpha-enolase, IL-18, and KLK-13 may be associated with an increase inflammatory response or peripheral nerve damage.[12]

Additionally, the increased absorbance in the spectral range between 2959 – 2876 cm^{-1} in the current study can possibly be associated with the upregulation of lipids which can be observed in the composition of hormones. Hormones such as progesterone, 17 β-estradiol, and DHEA were found to have a higher expression in BMS patients.[11,23] Moreover, cortisol is a hormone related to stress and BMS; interestingly, cortisol contains several CH_3, CH_2, and OH chains assigned in the range of 2959 – 2876 cm^{-1}. Increased salivary cortisol and higher anxiety levels were highly associated with BMS.[23,24] Increased cortisol may also influence gluconeogenesis which might

Table 2. Peak assignments as indicated by the normalized mean spectra plotted in Figure 1 [13,19,20]

Wavenumber (cm^{-1})	Vibration Type	Molecular Group
3273	ν NH	Amide A, nucleic acids
3078	CH stretching band of phenyl rings, $C_2 - CH_2$ aromatic stretching	-
2959	ν_{as} CH_3	Lipids, DNA, proteins
2934	ν_{as} CH_2	Lipids, fatty acids (methyl groups of mucous membrane lipids)
2876	Methylene asymmetric stretch of CH_2	Lipids
2112	ν SCN^-	Thiocyanates
2059	ν SCN^-	Thiocyanates
1638	ν CO	Amide I, proteins
1544	δ NH, ν CN	Amide II, proteins
1445	δ CH_2, δ CH_3	Methylene groups of sidechains of amino acids, lipids, and proteins
1403	ν_s COO^-	Fatty acids, amino acids
1243	ν_{as} PO	Nucleic acids, phospholipids
1071	C – O – C stretch	Sugars, phospholipids
926	ν CC, ν CO, ν CH_2OH	Sugars, glycosylated proteins
874	Left-handed helix DNA (Z form)	DNA
653	δ NH	Amide V
617	δ NH	Amide V
527	δ CO	Amide VI

Abbreviations: ν = stretching vibrations, δ = bending vibrations, s = symmetric vibrations and as = asymmetric vibrations.

be associated with the increased absorbance intensity observed at 1071 – 926 cm^{-1} relating to sugars and glycosylated proteins.[25]

Aside from the absorption bands observed in the spectral regions of interest, a slightly increased absorption intensity was also observed in 2112 – 2059 cm^{-1}, relating to thiocyanates. This finding is in line with the study of Rodrigues *et al*., which also noted a slight increase in thiocyanates in the salivary spectra in BMS.[13] Furthermore, an increased absorption intensity is also observed at 874 cm^{-1}, corresponding to bands of DNA, which might be related to increased microbial activity in the oral cavity. Reportedly, BMS has a negative effect on the oral health-related quality of life, in which the burning pain directly affects the patients' daily function,[26] which might relate to difficulty in adequate oral hygiene maintenance. This finding might also be connected to the lower pH as bacterial

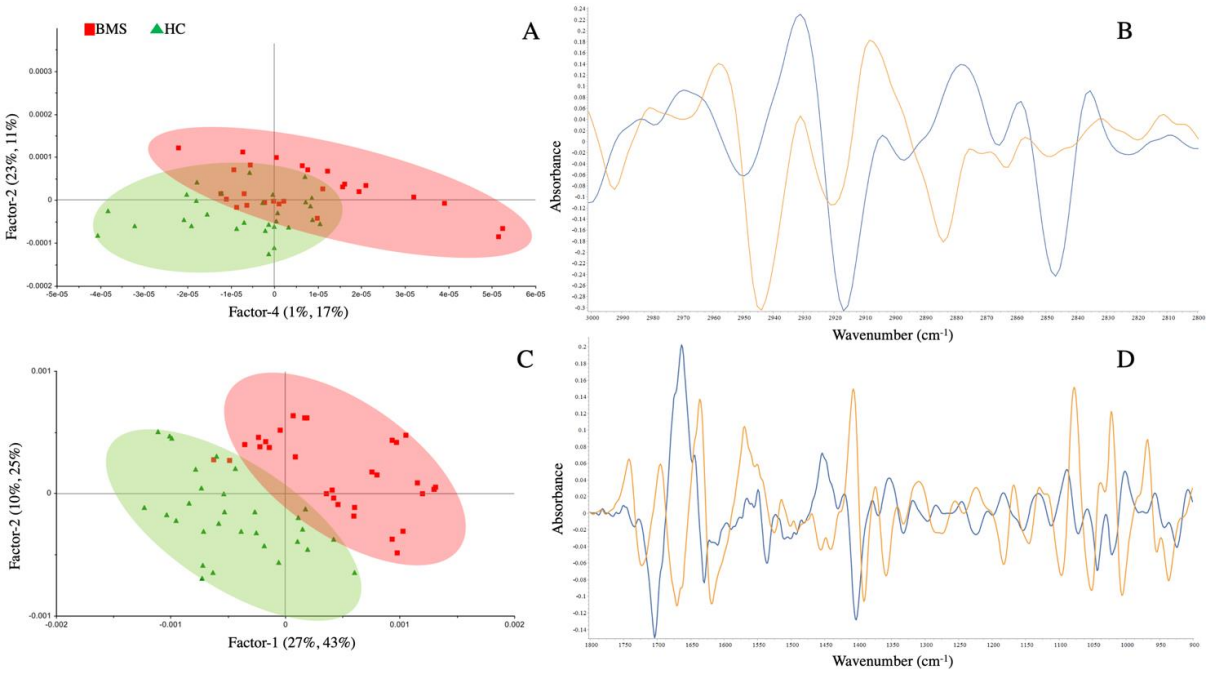

Figure 2. PLS scores plots (A, C) and corresponding loadings plots (B, D) of corrected average 2nd derivative spectra from the BMS (red) and HC (green) groups, showing discrimination between groups at Factor 2 (blue line) against Factor 4 (yellow line) at 3000 cm^{-1} to 2800 cm^{-1} (B), and at Factor 1 (blue line) against Factor 2 (yellow line) at 1800 cm^{-1} to 900 cm^{-1} (D).

byproducts tend to produce an acidic environment, and decreased USFR observed in the BMS group might affect the saliva buffering capacity.[27]

BMS is an enigma that still necessitates further investigation. More tests involving a larger sample size and correlation with other clinical symptoms might provide additional information and reinforce our findings. Likewise, other parameters such as saliva viscosity, stress scores, and pain scales might provide additional data for evaluation. A limitation of FTIR analysis is that it can only determine the presence of biomolecular groups without specific identification which necessitates further analyses to identify specific compounds.

Conclusion

The main objective of this study was to identify specific biochemical spectral signatures of saliva in patients with primary BMS, which may attest to changes in the salivary composition at a molecular level. ATR-FTIR spectroscopy and a supervised machine learning analysis helps in saliva characterization, providing a novel platform for disease diagnosis. The results of this study indicate an increased absorbance intensity for several salivary components in primary BMS patients. Although with the limitations of this study, biomolecular compounds of interest were identified serving as a stepping stone to more specific profiling, which may help understand the etiology of the disease and provide a more targeted approach to treatment of the disease.

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OM-00026

Dental Services Model for Older Adults in Pangmapha District: A Qualitative Study

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ABSTRACT

Background and Objective: Some remote areas have no dental personnel, which could worsen the problems. This study aimed to develop a model of dental services for older people in remote areas that lack dental personnel.

Methods: A qualitative research study conducted focus group discussions in 3 remote areas, including 33 participants. The two main themes were identified: What should the health of the older adults in the area be? and what are the service characteristics of the health promotion hospitals in the area expected by the participants? Data were coded, themed and analyzed abductively.

Results: The analysis revealed three themes emerging "the meaning of health, oral health and oral care with the quality of daily.", "Oral health service as a basic treatment at health promotion hospital." and "Oral health care competency of local health officers or village health volunteers are needed in the area without dental personnel."

Conclusion: Integrating the dental mobile services model with trained basic health screening of health care workers is essential for caring for older adults in rural areas.

Keywords: Dental services model, Health promotion hospitals, Older adults, Remote area

Introduction

Thailand as well, with 19 percent of the population aged 60 years and over, with an expectation that by 2022, it will enter a "Complete aged society." with 20% of older adults compared to total population.[1]

Older adults are more likely to have performance deterioration and health problems.[2] Regarding oral health, older adults are more prone to oral problems than other age groups.[3] This will affect the efficiency of mastication, quality of life and various systemic diseases that may follow. For this reason, self-care for oral health and access to dental services are essential. Older adults often lack access to dental services, especially those who live in remote areas.[4,5] Due to travelling problems, older adults have to rely on relatives or others to take care of or take them to the hospital. Therefore, a health promotion hospital, a health service near home, plays a vital role in caring for older adults for health.

Pangmapha district is a small district in Mae Hong Son province. It is an all mountainous area. Most of the population are ethnic tribes consisting of Tai-Yai, Red Lahu, Lisu, Black Lahu, Karen, Khonmeang, Lawa and Pa-O.[6] Pangmapha hospital is the central dental health service unit in the area. There are 5 health promotion hospitals in Pangmapha district as a downline network of Pangmapha hospital, of which four do not have full-time dental nurses.[7] Of these, three are located in remote areas that are difficult to travel. Due to the mountainous geography, health promotion hospitals in Pangmapha district are stand-alone. It is unable to transfer patients between health promotion hospitals easily.

This study was a qualitative part of the study "Dental services model for older adults of health promotion hospitals in Pangmapha district, Mae Hong Son province." The study aimed to develop a model of dental services for older adults in health promotion hospitals without dental nurses in remote areas.

Materials and Methods

Focus group discussions were conducted in 3 health promotion hospitals in Pangmapha district, Mae Hong Son province. All of them are located in remote rural areas. Participants were 4 community leaders, 10 village health volunteers (VHVs), 1 elderly caregiver, 12 leaders of the elderly in the area, 1 community health officer, 4 staff of health promotion hospital and 1 member of the subdistrict administrative organization (SAO).

A total of 33 participants were grouped into 3 focus group discussions, 28 males (84.8%), 5 females (15.2%), aged 25-83. Each participant provided written informed consent for IRB approval. The focus group instrument consisted of a structured interview guide. The researcher (TN) conducted the main discussion, and the staff of health promotion hospitals was the assistant. The audio was recorded throughout the conversation by 2 recorders.

The main questions in the discussion were addressed in the two areas: (1) What should the health of the older adults be? and (2) What are the service characteristics of the health promotion hospitals in the area expected by the participants? All the questions and answers were summarized at the end of the discussion.

After the discussion, verbatim transcripts were made from voice recordings. Data were then used to analyze the content. In content analysis, it was first to categorize main issues and then classify them by bringing together data elements related to the same content.

This study was approved by the Human Experimental Committee, Faculty of Dentistry, Chiang Mai University (No.16/2021).

Results

Theme 1: The "Health, Oral health and Oral Health Care" of older adults.

When asked about participants'views on how the health of older adults should be, they referred to health as a state in which they can spend their daily lives on their own, going to the farm or gardening generally without being a burden to their children. However, there was an idea of accepting the deterioration of the body with age.

"There was Uncle K and Grandfather J, who could always go to the fields. Both are in the 70s and still healthy and can ride a motorcycle." (Participants1. Focus Group 3.)

"Old Father L can do gardening alone." (Participants1. Focus Group 3.)

"Good health is to stay with children and grandchildren for a long time. It is a matter of the mind, ability to eat, not having a disease or anything like that." (Participants 8. Focus Group 3.)

The goals of local people's oral health are to be able to chew food, eat without difficulties and not have oral pain that affects daily life. There is also an opinion that older adults should regularly be cared for by dental personnel. Providing health information to older adults by their family members is not always possible, as discussed by the group.

"With having good teeth. You can eat whatever you want." (Participants 6. Focus Group 1.)

"I have teeth. I brush my teeth sometimes. I can chew." (Participants 4. Focus Group 1.)

"There are many people with no good enough tooth, having difficulty eating, sometimes unable to eat anything." (Participants 7. Focus Group 3.)

"I want a dentist to come here to check my teeth." (Participants 4. Focus Group 1.)

"There should be a health and teeth check once a year, including cleaning my mouth." (Participants 4. Focus Group 2.)

"If the elderly have health problems and a public health official recommends them to change health behavior, he will recognize and follow. I think the public officer is significant." (Participants 1. Focus Group 1.)

In terms of oral health care for older adults, the competency of VHVs is not adequate at this point.

"I think it's difficult. If assigning VHVs to help checking up oral health of the elderly. Because, at present, the work that VHVs have to do monthly is not yet complete. However, it is possible to train some volunteers to do this task." (Participants 10. Focus Group 1.)

Theme 2: Oral health service at health promotion hospitals.

The focus group discussion noted that if mobile dental services are not too far from the village, like services in health promotion hospitals, community leaders can transport the patients to the service area. However, it is more difficult to bring the older persons somewhere away, such as the hospital. Several aspects have been discussed, including expenses, language problems, and patient transport.

"If the dental services come to provide at health promotion hospitals, volunteers can take the elderly to receive services." (Participants 1. Focus Group 3.)

"If older adults have to come to the hospital, there will be several problems, such as financial problems, car expenses, food costs." (Participants 1. Focus Group 1.)

"Communication is the most difficult for the older adults here because they don't understand or know the language." (Participants 1. Focus Group 3.)

"I would like a dentist or a dental nurse from the hospital to provide basic dental services, which is important. It would be good to come to provide services 1-2 times a year." (Participants 1. Focus Group 1.)

The focus group discussion revealed a need for some available services, including promotion, prevention, and rehabilitation, provided by staff members. In this study, the participants complained about how often they needed to go to the hospital due to the services. Work may be integrated into routine tasks such as non-communication diseases (NCDs) or annual health checks for older people. This task seems not likely to be a burden on the staff of the health promotion.

"I did not go to the hospital but came to get medicine at a health promotion hospital. I went to the hospital once. At that time, two teeth were getting out. I was afraid that my teeth would run out." (Participants 5. Focus Group 3.)

"Some elderly people are edentulous. If he was going to have dentures, he had to go to the hospital several times before he could get the dentures." (Participants 2. Focus Group 3.)

"Let's come to have a dental check-up combined with the examination of all chronic disease patients because there is a regular check for this age group, at least once a year." (Participants 8. Focus Group 1.)

Theme 3: Oral health service at health promotion hospital in the case without dental personnel.

All participants expressed the need for oral health services in case no dentist or dental nurse. Preliminary screening should be conducted, providing information and ensuring that patients would be treated if they travel to the hospital. Initial assessment guidelines should be prepared for local officers of health promotion hospitals or VHVs to assess initial examination and provide information about dentures, health insurance, and patient expenses.

"We would like a referral form that health promotion hospital staff can pursue." (Participants 10. Focus Group 1.)

"I propose that VHVs be trained to perform simple oral screening. Moreover, the health record can use as referral information when going to the hospital or before the dentist come to our area." (Participants 1. Focus Group 1.)

In summary, all participants developed a model of oral health service for older adults, as shown in Fig. 1.

Discussion

The meaning of health could be different based on their background, experience and culture. For older adults in this study, the meaning of health is focused on well-being rather than disease-free, which is consistent with the definition given by the World Health Organization (WHO).[8] Although health declines with age, people should be encouraged that regular healthcare, exercise and good nutrition are essential to good health.

Older adults are more concerned about good oral function than disease-free. The concept complies with the proposed concept by The Japanese Society of Gerodontology (JSG), in which older adults need to be encouraged to have good oral function through the community process.[9] For further, the oral health program should focus more on oral function than older adult oral diseases.

Transportation is a significant obstacle in providing dental services in remote and mountain areas. Mobile services are essential for people in this area. Moreover, people should emphasise self-care and reduce reliance on experts. The integration of general healthcare and dental care, with aids of dental technology, such as Teledentistry[10], should be applied by the health personnel to help all ages, especially older people who are dependent on health services.

While communication can create a trend for the ethnic tribes to understand healthcare principles, it could be a barrier due to different languages. Community leaders, who could potentially be

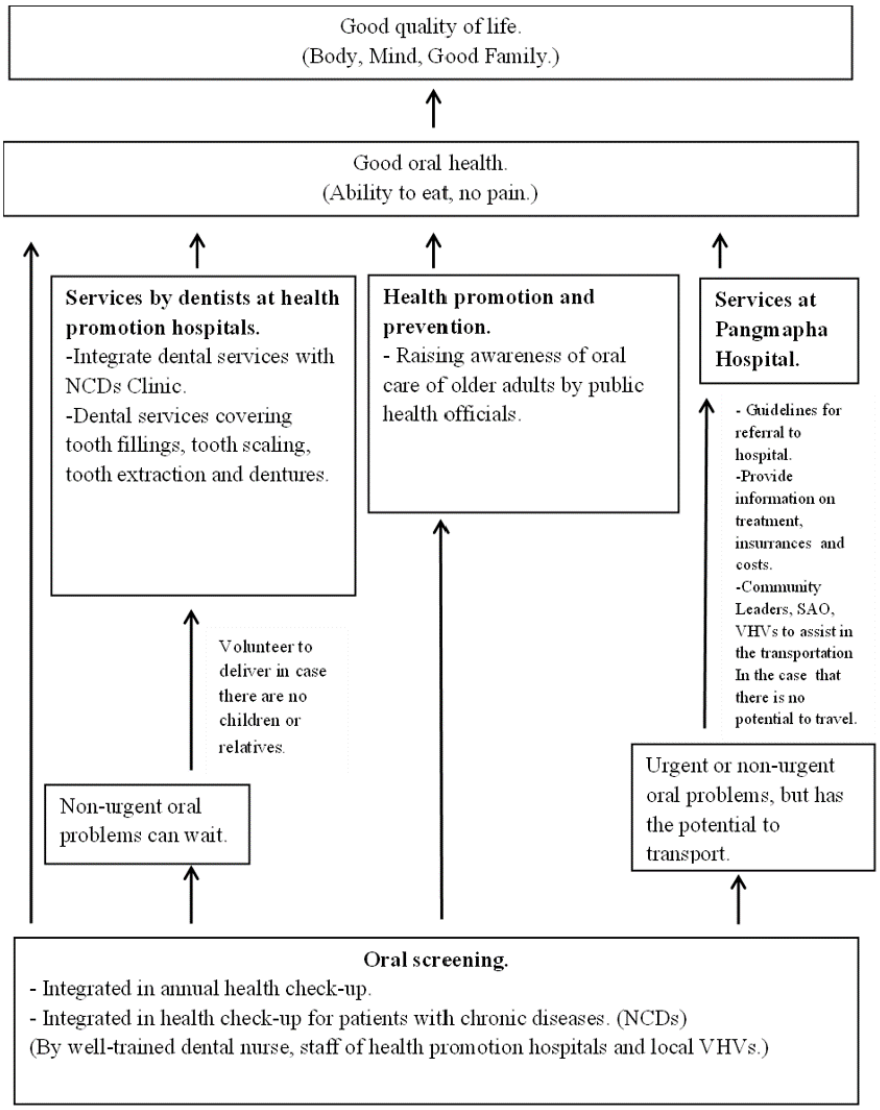

Figure 1. Oral health services model for older adults of Pangmapha health promotion hospitals.

young people, who can communicate in common languages and dialects, should help solve communication problems.

Simple oral health assessment guidelines, such as the Oral Health Assessment Tool (OHAT)[11], are accurate and effective. The health promotion hospital staff or volunteers should be trained to apply the guideline to assess oral health. The simple tools or questionnaires for an oral assessment are a good initiative for non-dental professionals.

Providing basic dental assessments that combine with existing services, such as NCD clinic, reduces the human resources required and can make appointments for older adults who are far from the meeting point to receive the service simultaneously. As a result, it reduces the hidden costs that people have to pay.

The model of dental service proposed by this study was achieved by brainstorming people who work in the area, practical in real situations. To reduce burdens, the model combines basic dental health care with existing health services, such as NCD clinics. The health promotion hospital works as a primary medical care setting. Moreover, the health promotion and oral disease prevention aspects need to be developed and made more effective.

However, as this study is a qualitative study, which was explained by the feelings and opinions from focus group discussion, a further quantitative study is needed to investigate whether the model is suitable for the area.

The finding of this study has limited generalizability as a few remote areas in the high mountain were selected, and the model of dental services for older adults is unique. It may not be suitable for urban areas with different cultures and beliefs. Therefore, implementing such a model requires an understanding of the context of the area.

Conclusion

This study suggested that the model of dental services without dental personnel in remote areas should provide mobile dental services with training in basic screening skills for health workers.

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OM-00027

Effects of Plant-Derived Epidermal Growth Factor on Salivary Gland Epithelial Cell Proliferation

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ABSTRACT

Background and Objective: Epidermal growth factor (EGF) is an important signaling cue for salivary gland (SG) development and is beneficial in preventing irradiation-induced xerostomia. Commercially available EGF is commonly produced via bacterial expression systems which lack post-translational modifications and can be costly and complex to manufacture. Recently, plant-derived EGF (P-EGF) has been developed to overcome these limitations. This study aimed to determine the role of EGF in SG epithelial branching at pre- and post-acinar stages and investigate the biological effects of P-EGF and B-EGF on SG epithelial cells.

Methods: Fetal submandibular gland (SMG) and sublingual gland (SLG) of ICR mouse embryos at embryonic day 13 (E13) and E16 were cultured with growth media supplemented with EGF (2 – 200 ng/ml). Glands were incubated for 24h and branching morphogenesis index was assessed by ImageJ software at baseline and 24h. Primary SG epithelial cells from *Sus scrofa* (pig) were isolated and characterized phenotypically, and the effects of B-EGF and P-EGF in a monolayer culture system were investigated. MTT and luciferase-based ATP assays were performed to determine proliferation of SG epithelial cells treated with B-EGF and P-EGF (5 – 20 ng/ml) at baseline, day 3, and day 6.

Results: EGF 20 ng/ml enhanced branching morphogenesis of the SMG and SLG at the post-acinar stage. In addition, B-EGF and P-EGF 5 – 20 ng/ml supported SG epithelial cell proliferation in a comparable fashion.

Conclusion: EGF supported SG branching morphogenesis at the post-acinar stage and epithelial cell proliferation. Moreover, P-EGF is comparable to B-EGF and may be a feasible alternative for B-EGF.

Keywords: Cell proliferation, Epidermal growth factor, Plant molecular farming, Salivary gland

Introduction

Salivary glands (SG) are essential exocrine glands that secrete saliva into the oral cavity. Saliva produced from SG plays an important role in preventing tooth decay, moisturizing oral mucosa, facilitating food digestion, and antimicrobial activity.[1] It is estimated that SG produces about 1 – 1.5 liters of total saliva volume a day, of which the unstimulated salivary flow rate (USFR) ranges from 0.3 to 0.4 ml/min, and the stimulated salivary flow rate (SFR) is between 1 and 2 ml/min.[2] Hyposalivation (also known as xerostomia) is diagnosed when USFR or SFR is less than 0.1 ml/min or 0.5 – 0.7 ml/min, respectively.[3] This condition leads to many undesirable effects, such as a high risk of dental caries, oral infection, and difficulties in chewing and swallowing.[4] Xerostomia is caused by various conditions such as medications, aging, head and neck radiation, Sjögren syndrome, and diabetes mellitus.[5] Although xerostomia is a common disease that affects 13.2% of the population, there has been no standard treatment.[6]

Patients suffering from xerostomia are commonly prescribed pharmaceutical drugs such as pilocarpine and cevimeline. These drugs have been approved by FDA (US Food and Drug Administration). Pilocarpine binds to M1 and M3 receptors as a muscarinic-cholinergic agonist.[7] Conversely, cevimeline is a muscarinic-cholinergic agonist that selectively interacts with M3 receptor.[8] Both drugs stimulate saliva secretion by interacting only with remnant secretory acinar cells in impaired-function SG. The main drawbacks of these two salivary stimulants are their numerous adverse effects, including sweating, gastrointestinal upset, flushing, and urinary issues. These adverse effects result in a 61% drug discontinuation rate in pilocarpine users and 32% in cevimeline users.[9] Therefore, a novel therapy with more potential and fewer side effects is required.

Epidermal growth factor (EGF) is an important signaling cue in SG epithelial development in the presence of basement membrane.[10] EGF is also a commonly added factor in the expansion media of SG stem and progenitor cells.[11] EGF receptor (EGFR)-deficiency mice exhibits impaired SG branching morphogenesis and delayed maturation of the SG epithelium.[12] Moreover, the inhibition of EGFR at early embryonic stage (E13) in the submandibular gland (SMG) leads to reduction of epithelial bud formation and an increase in mesenchyme apoptosis.[12] However, the role of EGF in SG branching morphogenesis and epithelial bud growth is poorly understood. Therefore, the first objective was to determine the role of EGF in SG epithelial morphogenesis and development at pre- and post-acinar differentiation stages in fetal murine *ex vivo* models.

Recently, Cho and colleagues reported that commercially available EGF is able to prevent irradiation-induced xerostomia by

protecting SG ductal and acinar cells from radiation damage.[13] Yet, commercial EGF is mainly produced via bacteria-based systems, which lack post-translational modifications and may carry toxins.[14] Recently, plant-derived recombinant human EGF (P-EGF) has been considered a feasible alternative to commercially available bacteria-derived recombinant human EGF (B-EGF) because of many factors including low-cost production, large scalability, low potential for human pathogen contamination, and the possibility to include post-translational modifications in the final plant-derived protein products.[15] Our research group has successfully produced a P-EGF from *Nicotiana benthamiana,* which is easily modified via genetic engineering and all year-harvested plant.[16] Our P-EGF has shown similar effect on proliferation of human skin epidermal keratinocytes when compared to commercial B-EGF.[17] The proliferative effects of this plant-based EGF biological product have not been studied yet in SG epithelial cells. Hence, as a second aim, this report investigated the biological effects of P-EGF and B-EGF on SG epithelial cells.

Taken together, this study has two objectives: (1) determine the role of EGF in SG epithelial morphogenesis at pre- and post-acinar stages; and (2) investigate the biological effects of P-EGF and B-EGF on SG epithelial cells.

Materials and Methods

Embryonic murine salivary gland *ex vivo* culture

All animal procedures were performed according to the approval of the Institutional Animal Care and Use Committee (IACUC) at the Chulalongkorn University Laboratory Animal Center (protocol number 1973004). This study was approved by the Research Ethics Committee and the Institutional Biosafety Committee of Faculty of Dentistry, Chulalongkorn University with reference numbers HREC-DCU 2022-019 and DENT CU-IBC 026/2021, respectively. Embryonic murine SG *ex vivo* culture was performed according to previous protocol.[18] A fertile inbred strain of female mice called ICR was purchased to retrieve the embryos and dissect the glands. In brief, fetal submandibular gland (SMG) and sublingual gland (SLG) were dissected from ICR mouse embryos at embryonic day 13 (E13, pre-acinar stage) and E16 (post-acinar stage) under a stereo microscope (SZH10, Olympus, Tokyo, Japan). Fetal glands were cultured on air-medium interface by placed on top of a porous polycarbonate membrane (Whatman Nucleopore, Sigma-Aldrich, St. Louis, MO, USA), which was floating on 200 µl growth media in a center well of 50 mm dish. Growth media composed of phenol-free Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12, Gibco, ThermoFisher Scientific, Waltham, MA, USA), 1% antibiotic-antimycotic (Gibco), 150 µg/ml ascorbic acid (Sigma-Aldrich), 100 µg/ml holo-transferrin (Sigma-Aldrich), and 2-200 ng/ml EGF (Sigma-Aldrich). Positive control (CTL) was growth media without B-EGF supplementation. This media has been established and validated in numerous SG developmental reports as “growth media” as it was shown to support the organogenesis of the SG.[18,19] Glands were incubated at 37 ºC, 5% CO_2 for 24h, and were observed under brightfield and phase-contrast microscopy at baseline and 24h. Number of epithelial buds at baseline and 24h were counted by ImageJ software (version 1.53s, NIH, Bethesda, MD, USA). Then, branching morphogenesis index (BMI) was computed by normalizing number of epithelial buds at 24h to numbers at baseline.

Isolation and of primary SG cells

Since mouse adult SG biopsies cannot provide large amounts of epithelial cell output for *in vitro* studies and human non-pathological SG tissue biopsies are not readily available in adequate quantities, this proof-of-concept study used porcine SMG since major SG of porcine share similar size, structure, and function with humans.[20] Moreover, porcine SG provide abundant epithelial cells after mechanical and enzymatic extraction.[21] The protocol of primary SMG cell isolation was thoroughly described in our previous report.[22] Briefly, SMG tissues were mechanically dissociated after removing connective tissues. Next, minced tissues were enzymatically digested within one hour by a digestion buffer composed of PBS, 1% antibiotic-antimycotic, 1% bovine serum albumin (Capricorn Scientific GmbH, Ebsdorf, Germany), 1.25 mM calcium chloride (VWR chemicals, Radnor, PA, USA), 0.4 mg/ml collagenase II (Gibco), and 0.7 mg/ml hyaluronidase (Sigma-Aldrich). Cells were collected by filtering consecutively through 100 µm and 40 µm cell strainers. These cells were then cultured in growth media including DMEM/F12, 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA), 1% antibiotic-antimycotic, and 1x L-glutamine (Hyclone). Primary cells were subcultured by using TrypLE Select (Gibco) when reaching 80% confluency. After first subculture, the concentration of FBS in growth media was reduced to 5% to limit potential contamination of fibroblasts. Morphology of SMG cells during cellular expansion from passage 1 (P1) to P3 was captured by an inverted light microscope (Leica DMi1, Leica Microsystems, Wetzlar, Germany). Cell viability was assessed by Trypan blue exclusion method.

Immunocytochemistry

Porcine SMG cells in single cell suspension from P1 to P3 were fixed with 4% paraformaldehyde (Sigma-Aldrich) for 10 minutes after trypsinization by TrypLE Select. Next, these cells were washed with PBS then blocked with blocking buffer composed of PBS, 10% horse serum (Gibco), 5% BSA, and 0.1% Tween 20 (Loba Chemie, Mumbai, India) for 2 hours. Blocked cells were incubated with anti-E-cadherin (Cell Signaling Technology, Danvers, MA, USA) at 4 ºC overnight. Then, these cells were washed three times, and incubated with Alexa 488 goat-anti rabbit (Abcam, Cambridge, UK) at room temperature for one hour. Nuclei was stained by Hoechst 33342 (Invitrogen, ThermoFisher Scientific). Fluorescent micrographs of immunostained cells were taken with Evos FL Auto II (ThermoFisher Scientific) and analyzed by CellProfiler software (version 4.2.1, Broad Institute, Cambridge, MA, USA).

Cell proliferation assay

Firstly, 1000 SMG cells (P1-P3) per well were seeded in 96-well plate for 24 hours with growth media composed of DMEM/F12 (Sigma-Aldrich), 3% FBS, 1% antibiotic-antimycotic, and 1x L-glutamine. After cells completely attached, media was replaced by fresh growth media supplemented with B-EGF or P-EGF (5 – 20 ng/ml). Control (CTL) was growth media without EGF supplementation. Following that, cells were incubated for 3 days and/or 6 days. To determine cell proliferation of SMG cells at each timepoint with different concentrations of B-EGF and P-EGF, an MTT assay (PanReac AppliChem, Darmstadt, Germany) and a luciferase-based ATP assay (CellTiter Glo 3D, Promega, Madison, WI, USA) was used following manufacturer instruction. For MTT assay, growth media in each well was discarded and cells were washed with PBS one time. Then 100 µl of MTT 0.5 mg/ml in growth media was added to each well and incubated for 2 hours. MTT solution was replaced by DMSO solvent to dissolve the formazan crystals prior to reading absorbance at 560 nm by Glomax Discover Microplate Reader (Promega).[23] For luciferase-based ATP assay, 125 µl of a solution containing CellTiter Glo 3D assay reagent and growth media with volume ratio 1:4 was added to each well after cells were washed with PBS. Plate was shaken in an orbital sharker (500 rpm) for 5 min and incubated at room temperature for an additional 25 minutes. Then Glomax Discover Microplate Reader was used to measure bioluminescence signal.[24] Values at day 3 or 6 were normalized to numbers at 2h to calculate fold change in cell proliferation.

Statistical Analysis

Mean and standard deviation (SD) was used to display all data in this study. One-way ANOVA with *Dunnett* or *Tukey post-hoc* analysis was performed to compare three or more groups. GraphPad

Prism software version 9 (GraphPad, San Diego, CA, USA) was used to perform all statistical analyses.

Results

EGF enhanced branching morphogenesis at the post-acinar stage in SMG and SLG

To investigate role of EGF at pre- and post-acinar stages of SG epithelial development and morphogenesis, murine SG (mSG) at E13 and E16 were treated with EGF 2 – 200 ng/ml for 24h. At pre-acinar stage, low levels of EGF (2 ng/ml) supported the BMI of the SMG and SLG when compared to CTL; however, EGF 20 and 200 ng/ml delayed mSG branching morphogenesis (Fig. 1A-C). At the post-acinar stage, B-EGF 20 ng/ml significantly increased the BMI of both SMG and SLG, whereas the BMI with B-EGF 2 and 200 ng/ml treatment was similar to CTL and supported epithelial branching (Fig. 2A-C). Moreover, extracted microarray data from the mouse Salivary Gland Molecular Anatomy Project (SGMAP) database[25] showed that *Egf* gene expression is downregulated at the pre-acinar stage (from E11.5 to E15) (Fig. 1D and E) and mainly expressed at the post-acinar stage (from E16 onwards) in both SMG and SLG (Fig. 2D and E). These findings suggest that EGF promotes epithelial branching morphogenesis of both the SMG and SLG, particularly at the post-acinar stage when acinar cells in the endbuds start the differentiation process.

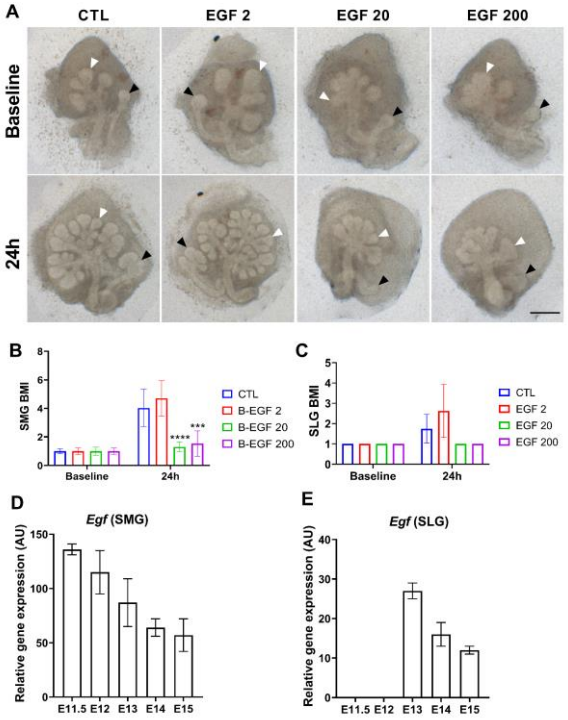

Figure 1. Low EGF levels supported epithelial morphogenesis at the pre-acinar stage in murine SMG and SLG. (A) Brightfield micrographs of E13 SMG (white arrowhead) and SLG (black arrowhead) cultured with EGF (2 – 200 ng/ml) for 24h. Mag.: 4x, scale bar: 50 µm. Quantification of branching morphogenesis index (BMI) of SMG (B) and SLG (C) by normalizing number of epithelial buds at 24h to numbers at baseline. Data are displayed as mean ± SD from n = 5. Relative gene expression of *Egf* at the pre-acinar stage (E11.5 – E15) in SMG (D) and SLG (E) was extracted from the SGMAP database.[25] *** p<0.001, and **** p<0.0001 when compared to CTL by using one-way ANOVA with *Dunnett post-hoc* test.

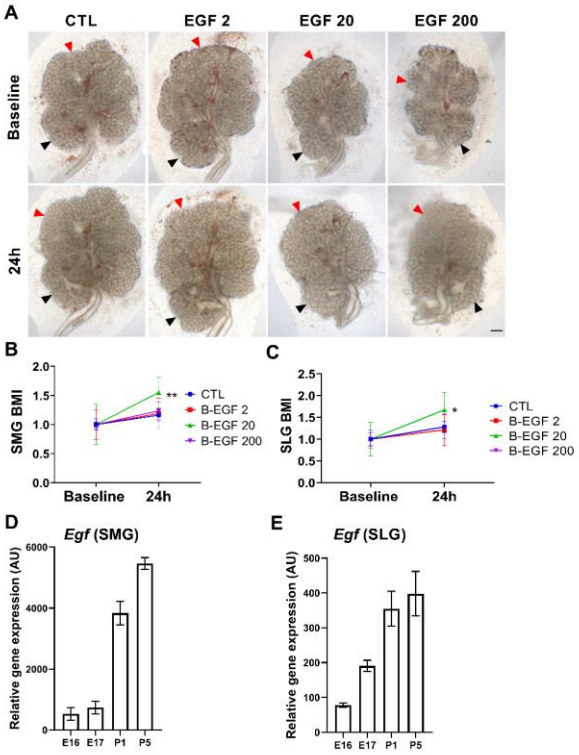

Figure 2. EGF 20 ng/ml promoted branching morphogenesis at the post-acinar stage in murine SMG and SLG. (A) Brightfield micrographs of E16 SMG (red arrowhead) and SLG (black arrowhead) cultured with EGF (2 – 200 ng/ml) for 24h. Mag.: 4x, scale bar: 200 µm. Quantification of branching morphogenesis index (BMI) of SMG (B) and SLG (C) by normalizing number of epithelial buds at 24h to numbers at baseline. Data are displayed as mean ± SD from n = 5 – 10. Relative gene expression of *Egf* at post-acinar stage (E16 – P5) in SMG (D) and SLG (E) was extracted from the SGMAP database.[25] * p<0.05 and ** p<0.01 when compared to CTL by using one-way ANOVA with *Dunnett post-hoc* test.

Isolated SG cells maintained an epithelial phenotype from P1 to P3

There were two distinct populations of primary SG cells based on morphology, including epithelial-like cells (black arrowhead in Fig. 3A) and spindle shape-like cells (white arrowhead in Fig. 3A). E-cadherin (E-CAD) is a specific marker for epithelial cells.[26] Within the first three passages, there was no difference in the percentage of E-CAD+ cells (Fig. 3B and C). In addition, cell viability was maintained from P1 to P3 (Fig. 3D). Previous study of our group has shown that the spindle-shaped cells (which are in low numbers in Fig. 3A) express smooth-muscle actin, a marker for myoepithelial cells.[27] These cells are present in the native SG surrounding the acinar cells and are part of the epithelial compartment.[28] These findings indicates that primary SG cells exhibited an epithelial phenotype from P1 to P3, and hence these passages were used for subsequent experiments.

P-EGF and B-EGF had comparable effects in SG epithelial proliferation

To determine optimal concentration of B-EGF and P-EGF suitable for an *in vitro* SG epithelial cell culture system, an MTT assay was used to measure the proliferation of SG-derived epithelial cells treated with different concentrations of B-EGF or P-EGF (5 – 20 ng/ml), as displayed in Fig.4 and 5, respectively. The MTT assay showed that both B-EGF and P-EGF supported epithelial cell proliferation. B-EGF only increased SG epithelial cell proliferation after 6 days of culture (Fig. 4A and B); whereas P-EGF enhanced proliferation at day 3 and 6 (Fig. 5A and B).

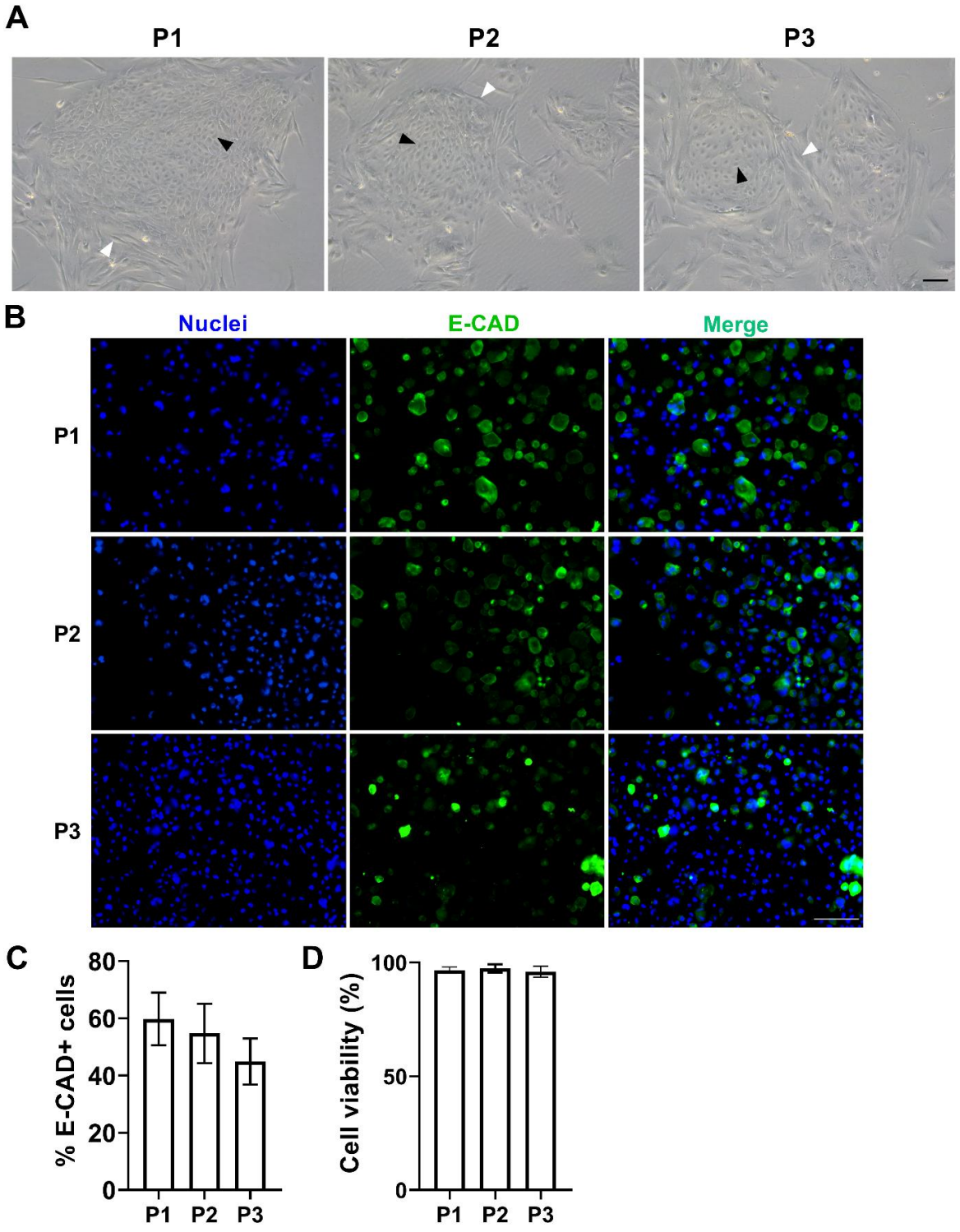

Figure 3. Isolated SG cells maintained epithelial phenotype from P1 to P3. (A) Representative brightfield micrographs of SMG cells from P1 to P3. Epithelial-like cells (black arrowheads) decreased in number from P1 to P3. Spindle shape-like cells are indicated with white arrowheads. Mag.: 10x, scale bar: 100 µm. (B) Representative fluorescence micrographs of suspended SG cells from P1 to P3, which were immunostained with marker for epithelial cells (E-CAD). Mag.: 20x, scale bar: 100 µm. (C) Quantification of E-CAD+ cells by normalizing with total cell counts by CellProfiler software version 4.2.1. Data are displayed as mean ± SD from n = 5. (D) Cell viability from P1 to P3 was assessed by Trypan blue exclusion method. Data are displayed as mean ± SD from n = 3.

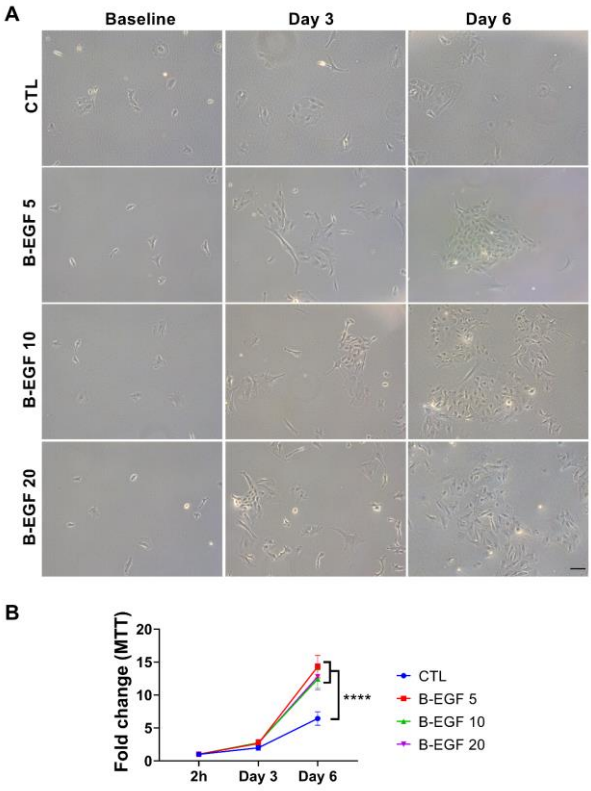

Figure 4. B-EGF 5 – 20 ng/ml supported SG epithelial proliferation. (A) Representative brightfield micrographs at baseline, day 3, and day 6 after treatment with different concentrations of B-EGF while comparing to CTL (without B-EGF). Mag.: 10x, scale bar: 100 µm. (B) The proliferation of SG epithelial cells treated with B-EGF (5 – 20 ng/ml) on day 3, and day 6 was determined by MTT assay. Optical density (OD) values on day 3 and day 6 were normalized to OD values at baseline. Data are displayed as mean ± SD from n = 6. **** p<0.0001 when comparing each group to CTL (without B-EGF) by two-way ANOVA with *Dunnett post hoc* analysis on day 3 and day 6.

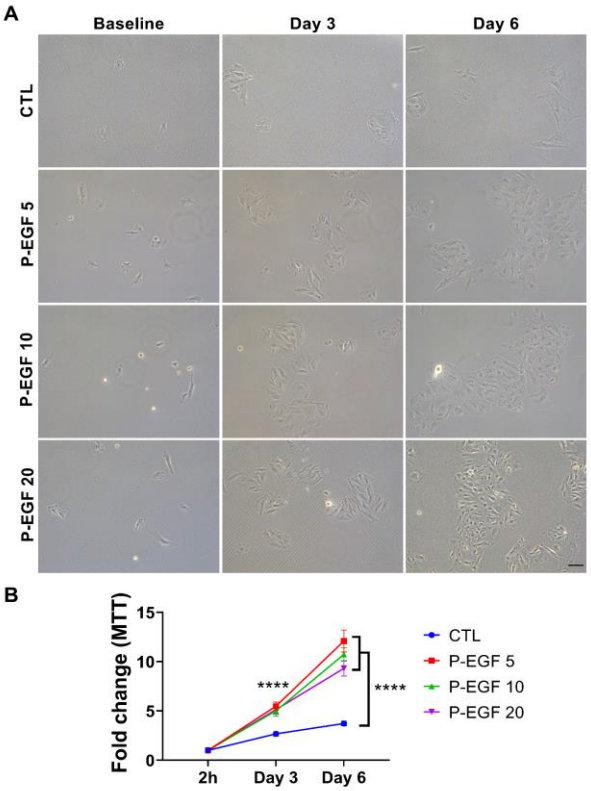

Figure 5. P-EGF 5 – 20 ng/ml supported SG epithelial proliferation. (A) Representative brightfield micrographs at baseline, day 3, and day 6 after treatment with different concentrations of P-EGF while comparing to CTL (without P-EGF). Mag.: 10x, scale bar: 100 µm. (B) The proliferation of SG epithelial cells treated with P-EGF (5 – 20 ng/ml) on day 3, and day 6 was determined by MTT assay. Optical density (OD) values on day 3 and day 6 were normalized to OD values at baseline. Data are displayed as mean ± SD from n = 6. **** p<0.0001 when comparing each group to CTL (without P-EGF) by two-way ANOVA with *Dunnett post-hoc* analysis on day 3 and day 6.

Next, a luciferase-based ATP assay was utilized to compare P-EGF with B-EGF (Fig. 6). B-EGF 5 ng/ml was selected as the optimal concentration for comparison with P-EGF. The outcomes revealed that P-EGF 5 – 20 ng/ml was comparable to B-EGF 5 ng/ml in SG epithelial cell proliferation (Fig. 6A and B). These findings suggest that both P-EGF and B-EGF supported the proliferation of SG epithelial cells, and P-EGF exhibited similar biological effects in epithelial proliferation as B-EGF.

Discussion

Firstly, our fetal SG culture model demonstrated that EGF 20 ng/ml enhanced epithelial bud formation and branching morphogenesis at the post-acinar stage but not at the pre-acinar in both the submandibular and sublingual glands. Interestingly, low EGF (2 ng/ml) slightly increased branching morphogenesis of SMG and SLG at the pre-acinar stage. Whereas moderate EGF levels (20 ng/ml) and high EGF levels (200 ng/ml) delayed bud formation in this stage. These outcomes are supported by the SGMAP database[25] where *Egf* is downregulated in embryonic SG from E11.5 to E15. However, *Egf* gene expression is gradually upregulated since E16 and is highly expressed in the post-natal mouse SMG. A previous report by Jaskoll and colleagues also found that TGF-α (not EGF) was a ligand of EGF receptor (EGFR) at the pre-acinar stage, which indicates that EGF may not be the key signaling cue modulating branching morphogenesis at earlier stages of SG development.[29]

Next, primary SG cells were successfully isolated and expanded, which maintained epithelial phenotype within first 3 passages as well as cell viability. Porcine SG cells were used due to sexual dimorphism found on mouse SG transcriptomes and ductal phenotype, which are not seen in human SG. Taro and colleagues reported a significant difference at the transcriptome level between male and female mice. Moreover, each major SG expresses different level of sex-specific genes, which is identified as SMG, SLG, and PG in descending order.[30] The *in vivo* porcine SG have also been used as a translational model to investigate efficacy and safety of neurturin gene therapy in the prevention of irradiation-induced hyposalivation since porcine and human SG share similar anatomy, physiology, and tolerate the same dose of irradiation.[31]

Regarding the biological effects of bacteria- and plant-derived EGF on SG epithelial proliferation, B-EGF and P-EGF ranging from 5 to 20 ng/ml promoted epithelial proliferation on a similar model. Interestingly, both *ex vivo* and *in vitro* models showed that EGF did not work on a dose-dependent manner. Specifically, very high levels of EGF do not increase branching morphogenesis and SG cell proliferation when compared to very low concentrations. This biphasic dose phenomenon is called “hormesis” and is characterized by inhibitory effects at high drug dosages instead of stimulatory ones.[32] A biological reason that may explain this finding is that the fetal SG at post-acinar stage produces endogenous EGF levels from the surrounding mesenchyme and epithelia[25], which together with the high exogenous EGF may lead to a condition of environmental stress for the cells triggering a negative feedback loop like an adaptive cell response towards overcompensation, culminating in the inhibition of epithelial bud growth.[33]

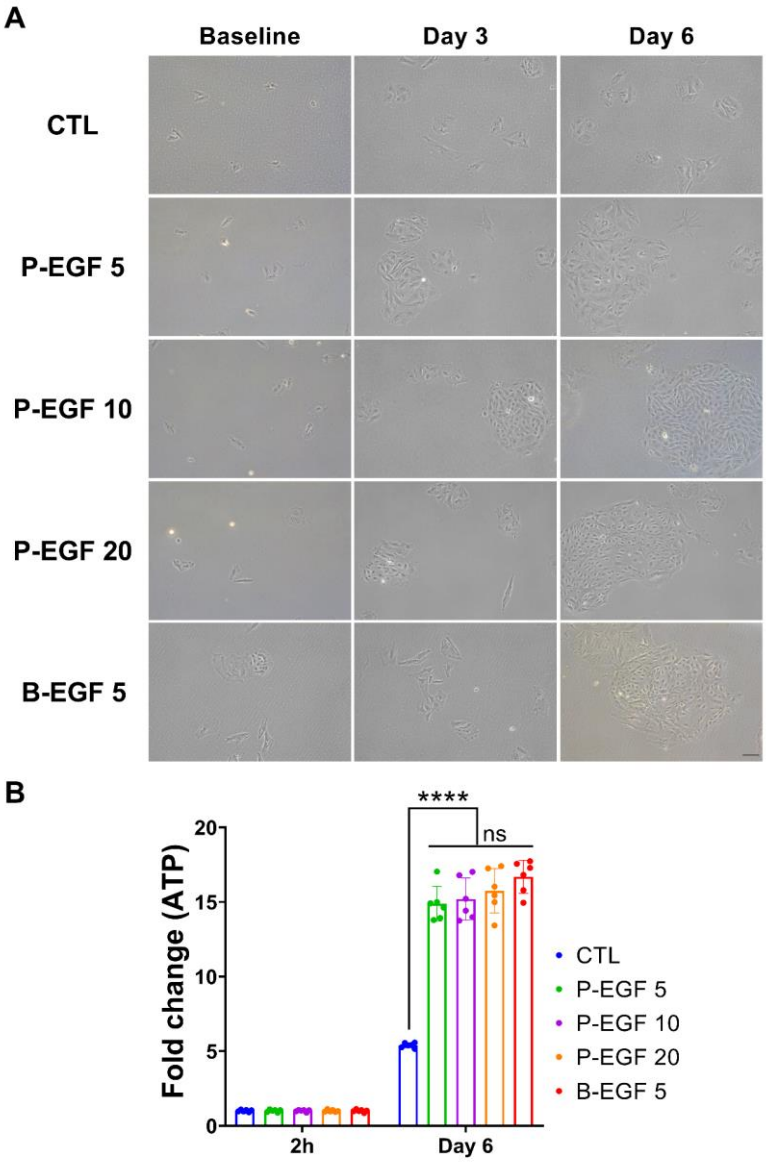

Figure 6. B-EGF and P-EGF promoted the proliferation of SG epithelial cells for 6 days on a comparable fashion. (A) Representative brightfield micrographs at baseline, day 3, and day 6 after treatment with different concentrations of P-EGF while comparing to CTL and B-EGF 5 ng/ml. Mag.: 10x, scale bar: 100 µm. (B) The determined optimal concentration of B-EGF (5 ng/ml) was used to compare with P-EGF treatment at different concentrations by using a luciferase-based ATP assay. Data are displayed as mean ± SD from n = 6. ns: not significant, **** p<0.0001 when used a one-way ANOVA with *Tukey post hoc* analysis.

B-EGF has been widely used in the growth and expansion media of primary SG cells.[11,34] Still, to our knowledge, this is the first study investigating the biological effects of P-EGF on SG epithelial cells. Our findings are similar to previous studies where P-EGF was comparable to B-EGF in the proliferation of kidney epithelial cells and human skin epidermal keratinocytes.[17,35] These effects may be explained by the same affinity of both EGF products to EGF receptor.[36] Interestingly, P-EGF enhanced SG epithelial cell proliferation at day 3, whereas B-EGF only did such at day 6. This is possibly due to the presence of post-translational modifications in plant-based expression systems, which lacks in bacteria-based systems.[37] Recently, Loo et al has found pB1, a plant-derived agonist of EGFR from *Pereskia bleo*. This is a low-affinity agonist of EGFR; however, its analog, [K29k]pB1 increases 60-fold affinity compared to pB1 and 100-fold stability compared to B-EGF. [K29k]pB1 and B-EGF have shown a comparable effect on wound healing process in diabetic mice model.[38]

This study compared SG epithelial proliferation using a simplistic monolayer culture system, which does not well recapitulate the multidimensional *in vivo* environment. Therefore, future studies will be conducted to assess biological effects of B-EGF and P-EGF in three-dimensional (3D) culture platforms. Moreover, further experiments will be performed to investigate which epithelial cells are promoted by EGF and what EGF and non-EGF receptors are targeted.

Conclusion

In summary, this study found that EGF mainly enhanced or supported epithelia branching and morphogenesis at post-acinar developmental stages in the mouse SMG and SLG. Moreover, B-EGF and P-EGF 5 – 20 ng/ml supported SG epithelial cell proliferation on a comparable fashion. Our outcomes suggest that P-EGF is a feasible alternative for B-EGF in SG epithelial proliferation. Future studies are

ongoing to compare P-EGF and B-EGF in 3D culture platforms for SG organoid formation.

Acknowledgements

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Patients' Perception of Dental Treatment during COVID-19 Pandemic: Qualitative Interview

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ABSTRACT

Background and Objective: Dental treatment has been affected from the COVID-19 outbreak since the airborne droplets and aerosols generated from intraoral operation may transmit the viral particles. During the outbreaks, many dental patients expressed their anxiety and quit their dental appointments. It is needed to adjust the dental treatment according to the new normal after this pandemic. The aim of this study was to explore the attitudes, concerns, and behaviors of Thai people towards dental treatment during the COVID-19 outbreak.

Methods: The qualitative interviews were performed in 10 participants during January and February 2022. The questions consisted of 3 parts: demographic data, COVID-19 perceptions, and the attitudes toward COVID-19 and dentistry. The audio records were transcribed, and thematic analysis was used to analyze the data.

Results: Most of the participants had a significant reduction in anxiety and fear of contracting COVID-19 during the Omicron period comparing to the earlier variants. Two participants had their dental health checked but less frequent than before the pandemic. Seven participants had their dental symptoms treated during the outbreaks. Most of them felt safe from COVID-19 infection because of COVID-19 preventive measures. While some of them still concerned of contracting infection due to the need of mask removal during dental treatments or doubted the infection control methods.

Conclusion: Most of the Thai dental patients in this study had their dental problems treated with COVID-19 preventive measures. They felt confident in their dentists and clinics with some concern about contracting COVID-19 through dental work.

Keywords: Attitude, Behavior, COVID-19, Dental patients, Dental treatment

Introduction

Coronavirus disease (COVID-19) is an infectious disease caused by virus known as SARS-CoV-2. The first COVID-19 outbreak began in Wuhan, China, in December 2019 and then it has rapidly spread throughout the world.[1] The transmission route of SARS-CoV-2 are mainly direct contact and droplet transmission. Aerosol transmission is also possible in place that exposure to high concentrations of aerosols such as closed environment.[2] Many reports suggest that SARS-CoV-2 can be found in the saliva and salivary gland of infected patients.[3–7] Meanwhile, dental treatment procedures can generate airborne droplets and aerosols which contain patients' saliva, blood, microorganisms, and other debris. Thus, it has a chance that SARS-CoV-2 may spread during dental treatment by SARS-CoV-2 infected dental patients.[8]

During the disease outbreak, elective medical health care especially aerosol-generating procedures including dental operations are postponed reducing the chance of disease transmission. Many dental organizations launched guidelines for dental treatment during this pandemic such as patient triage, preoperative mouthwash, Personal Protective Equipment (PPE) application, or adequate room ventilation.[9,10] Moreover, patient's anxiety of contracting COVID-19 during dental treatment was reported [11-14] and it was associated with the decision to get dental treatment.[11] In the early phases of the outbreak, a research study was being conducted in Thailand's Kalasin Province.[15] However, there hasn't been any research on Thai patients' perceptions of getting dental treatment after having to deal with the coronavirus outbreak for a while. The aim of this research is to explore the attitudes, concerns, and behaviors of Thai people towards dental treatment during the COVID-19 outbreak by in-depth interview.

Materials and Methods

This research was a qualitative study whose sample selection method was convenience sampling. A total of 10 participants who could understand and speak Thai clearly, lived in Thailand during the COVID-19 pandemic, and consented for an interview after obtaining ethical approval (HREC-DCU 2021-091).

The questions for the qualitative interview were semi-constructed with open-ended questions, which consisted of 3 parts: demographic data, perceptions about COVID-19, and COVID-19 and dentistry. All questions were validated from 3 experts in the field of dentistry.

The qualitative interviews were carried out during January and February 2022, which was the early stages of the Omicron variant outbreak in Thailand. Interviews were conducted via Line, Messenger, or phone call, that depends on the convenience of the interviewee, and recorded using the Stereo IC Recorder ICD-PX470 SONY®. The duration of the interview was approximately 30–45 minutes. At the end of the interview, all participants would receive a payment of 500 baht.

The audio records were transcribed word-for-word (Transcription of Data). The data collection was done systematically

by categorizing the words or messages through which the participants expressed their opinions. Then, interview data was stored on a protected server and accessible only by the research team. Thematic analysis was used to analyze the data.

Results

Ten participants, who resided in Thailand during COVID-19 pandemic, were recruited. The demographic characteristics of sample are presented in Table 1.

Table 1. The demographic characteristics.

Characteristics	Number of Participants
Gender	
Male	5
Female	5
Age (year)	
<20	1
20-29	4
30-39	2
40-49	1
50-59	2
Medical status	
Healthy	8
Allergy	1
Hypertension, diabetes, hyperlipidemia	1
Region	
Capital city (Bangkok)	5
Provincial city	5
Monthly income (Baht)	
< or 5000	4
5001 – 15000	2
15001 – 25000	2
25001 – 50000	2
Education level	
High school or less	1
College/university or diploma	6
Master's degree or higher	3

Nearly all the participants had been COVID-19 vaccinated (2 or 3 doses), only one was not vaccinated because she concerned about side effects. One participant was infected with COVID-19 while 4 participants had infected close relatives. Facebook, television, and Line were the three main media sources for COVID-19 news.

Perception about COVID-19

Most of the participants in the first phase of the pandemic were scared and worried about the coronavirus. Some people didn't even dare to leave the house or go to a place where there were a lot of people, especially before the COVID-19 vaccine. One of the participants was also concerned about getting RT-PCR testing, which was expensive and difficult to access at that time. However, most of the participants had a significant reduction in anxiety and fear at the period of this interview because of the virus mutation and COVID-19 vaccination resulting in milder and less lethal symptoms of the infected patients. The knowledge and social measures to protect themselves made them more confident and able to adapt to a new way of life. In addition, most participants weren't worried about the severity of their symptoms or their chances of dying from COVID-19 because they believed they were healthy. Nevertheless, there were still some concerns because the Omicron variants in this period could be transmitted much more easily. Therefore, they concerned that they could be a virus carrier to their families such as young children or the elderly. They also worried that the infection could affect their study or work because of the quarantine period for COVID treatment.

The concern also depends on the career, especially in occupations that require them to meet a lot of people. For example, a participant who had part-time work as a waiter in a restaurant at night or another participant who was a primary school teacher said that she had to meet many students and thought that the protection of the children might not be good enough. However, one participant, who had a career as a salesperson with 20–30 customers to meet each day, had a different attitude. He said, "I no longer fear this disease because of the experience that I have had with it before, which made me feel that it wasn't scary at all."

COVID-19 and Dentistry

During COVID-19 pandemic, 7 participants had oral health care behaviors by brushing their teeth twice a day and 3 participants brushed after meals. Three participants had better oral hygiene behaviors than before the pandemic, for example, 1 participant brushed the teeth twice a day (morning and night) instead of once in the morning, and 2 participants brushed after meals instead of twice a day. Some participants used adjunctive care products like dental floss and interdental brushes during the coronavirus outbreak as their dentists' recommendation.

Nine participants received dental care during the COVID-19, including 7 participants who had symptoms of dental disease, and 2 participants who had routinely dental checked up once a year. Prior to the COVID-19 outbreak, those who had routine dental checkups had them twice a year on average. One participant reported that the dental office was closed during the first year of the COVID-19 outbreak, barring him from receiving a routine tooth examination. Another person missed the dental practice his first year because he was so nervous about COVID-19. Among those who received dental treatment during the pandemic due to symptoms like toothaches, fractured teeth, bad breath, problems with tooth restoration, or a need for orthodontic treatment. Most of the patients received dental treatment. Only one person in this study who was canceled from the hospital owing to the COVID-19 situation, did not go to any dental checkups at all.

There are many different dental treatment locations for participants, such as private clinics, government hospitals, private hospitals, the Faculty of Medicine Hospital, and the Faculty of Dentistry. The factors in choosing a place to receive dental care for patients during the COVID-19 pandemic can be summarized as follows (One person can answer many factors) : Good service from dentists and staff (6 people), dental treatment costs were not too expensive (5 people), cleanliness of the clinic (3 people), standard quality of treatment (2 people), not crowded patients or those who had an appointment in advance, so patients did not have to sit and wait for a long time (2 people), and a clinic near their home (2 people).

Most of the patients (7 people) who came to receive dental treatment during the COVID-19 outbreak were confident that they would be safe from COVID-19 infection because they noticed that the clinic looked clean and had a patient appointment in advance. The dentists and staff were dressed up properly, which made them quite believe that they would not get infected by the authorities. Having mouthwash before dental treatment, an isolated dental treatment room, and changing new tools for each patient were also factors for their confidence. While the waiting area was at a higher risk of getting infection because they had to sit with others. As one participant commented, "I was more afraid when I was sitting and waiting because I saw people sitting next to each other, holding mask, and touching their noses. In the dental room, I was not afraid because I thought that the staff had to clean first."

People who were still more concerned about contracting infection from dental treatment because, while receiving treatment, they had to remove the mask. Some of them doubted the cleanliness of dental tools or dental chairs, which may be the transmission route of infection from the previous patient. A participant, who received

dental care in the hospital, thought that there was a greater chance of contracting COVID-19 from other patients than dental work.

Regarding ATK testing prior to dental treatment, there were a variety of viewpoints. According to the first group, if both patients and dental personnel underwent ATK testing, it was beneficial and boosted confidence for everyone. Some participants claimed that since patients were likely to be COVID-19 carriers, dental authorities would benefit if only patients underwent ATK testing. As a result, the clinic should pay for the ATK test, and the patient may have to pay some fees, but the ATK test should be free at a government hospital. Additionally, one participant advised that the ATK test be kept running until COVID-19 has a known therapy or the symptoms are milder, like the common cold. On the other hand, one participant felt that the ATK test was not required since there was no risk of disease transmission or because there were few people present when she went to the dentist at the appointed time.

As the number of COVID-19 preventive measures increased during this period, hospitals and private clinics had to spend more money to improve systems or items, which caused many places to collect more treatment costs or extra costs than before. From the perspective of the patients, it was discovered that some of them were willing to pay the additional expense for their safety. Two people claimed that, despite having agreed to pay, they were unaware of the equipment or additions. Another group disagreed because they did not see other obvious COVID-19 preventive measures, which they believed should have been taken prior to the COVID outbreak given the existence of other respiratory illnesses like colds. Another group also believed that because dental treatment costs are typically high, they should not be charging patients for additional costs.

Discussion

According to the interview findings, most patients had better oral health care habits. This could be because most of them experienced dental symptoms during the coronavirus outbreak. As a result, there was a greater awareness of oral health care.

Most of the patients had low anxiety about contracting COVID-19 from dental treatment because the outbreak in this period (Omicron variants) had mild symptoms, and most of them were among the group who dared to receive dental treatment. If compared to another qualitative study in Thailand, which was conducted in an early pandemic, the group of people who did not receive treatment would be a group that was highly concerned.[15] Therefore, additional data should be collected among those who did not receive treatment during the Omicron outbreak.

Additionally, more patients received dental services during this period because dental guidelines began to relax. This enables the clinic to continue providing treatment as usual in accordance with COVID-19 preventive measures. In contrast, numerous international studies[11-14] claimed that most patients would not receive treatment because the clinic was closed or only provided urgent or emergency care in the early stages of the epidemic.

Although most people trusted dentists and clinics, they would take precautions and keep to the standards. On the contrary, some patients had questions about this matter. The public relations of patients with knowledge and understanding about the prevention of COVID-19 in the dental clinic may enable the patient to reduce anxiety and have confidence in returning to continue receiving services.

Conclusion

During the early Omicron variant COVID-19 outbreak, most Thai patients who participated in qualitative interviews presented better oral health care behaviors. Most of them already have dental care with COVID-19 preventive measures because they had oral health issues. Most patients had confidence in their dentists and clinics, so they were not very concerned about contracting COVIDS through dental work.

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Need Analysis for Thai Dental Assistants in Professional Development

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ABSTRACT

Background and Objective: The competencies of dental assistants (DAs) are common but there are some differences in some competencies depending on context and necessities. The purposes of this research were to analyze and identify the performance gaps and the priority needs of the performance gap of Thai DAs to meet dental professionals' expectations.

Methods: This research was conducted with the dual-response 5-Likert's scale questionnaire. The content validity test of questionnaire was 0.67-1.00 while the reliability test was 0.957-0.975. The questionnaire was distributed by emails and Line application to 764 dentists and DAs in Thailand. The performance gap was analyzed by the difference of expected and current performance. The priority needs of performance gaps were analyzed by a modified priority need index. The population of this research is the dentists and DAs in Thailand, approximately 38,000. The sample size from Yamane's calculation was 396 and included the dropout was 764.

Results: The response rate was 56% (428/764) which consisted of 213 dentists and 215 DAs. The 3 highest performance gaps and priority needs were similar which were 1) "competently perform basic life support in line with current guidelines", 2) "support dentists for dental health promotion in the community", and 3) "demonstrate knowledge of and ability to perform first aid for common minor accidents and condition", respectively.

Conclusion: This research demonstrates the performance gaps in the professional development of Thai dental assistants, which would be the basis of systematic improvement of dental assistant training programs.

Keywords: Dental assistant, Need analysis, Performance gap, Priority need, Professional development

Introduction

Dental assistants (DAs) are one of the dental auxiliary professionals who play an important role to help and support dentists for oral health care. The professional competencies of DAs are summarized from many national standards. They are generally categorized as dental assisting competencies,[1-3] health promotion and disease prevention,[1-3] community involvement,[1,3] patient care,[1-3] professional growth and development,[1] management.[3] The common competencies are similar among accredited programs but there are some differences in some competencies depending on context and necessary.

The dental practices consist of many complex and sensitive procedures that make dental treatments into a high-risk situation. These adverse and near missed events related to DAs competencies have a high impact on patient care and safety. The professional competencies of DA should be investigated for the causes of errors and improved for performance discrepancies.

These raises the question of which competencies are needed of professional development of Thai DAs. Are current competencies enough for DAs to work efficiently nowadays and the future? To answer this question, the performance gap should be clarified. The need analysis will explore the differences between the expected performance and the actual performance. This research aimed to analyze and identify the performance gaps of Thai DAs and the priority needs of the performance gap for Thai DAs to meet dental professionals' expectations.

Materials and Methods

This research was conducted as survey research.

Sample

The population of our need analysis study was registered Thai dentists who were the members of the Dental Council of Thailand and working in dental profession, and DAs who were assisting dentists on dental services in Thailand. Registered dentists to the Dental Council in Thailand and still alive were 18,234 dentists in 2021 (unpublished data). There was no statistical record of number of DAs in Thailand. The number of DAs was estimated by presuming one dentist needs, at least, one DA to perform dental services. Therefore, the number of DAs in Thailand was approximately 19,000. The total number of the populations of need analysis in this research was about 38,000. The sample size was determined by using Yamane's equation with the level of precision as 0.05. The sample size from calculation was about 396. Then the approximation of the response rate in this research was 53% as the result from Baruch and Holton study.[4] Thus, 764 questionnaires were distributed. The multi-stage sampling was used to select sample.

Questionnaire

The questionnaire was constructed by reviewing literature in DAs' and healthcare professionals' competencies. DA's competencies were from American Dental Education Association, General Dental council, Thailand Professional Qualification Institute, Faculties of Dentistry. The healthcare professional competencies were from soft skills, WHO's life skills and non-technical skills (NT). This research used dual-response questions (the current performance and the expected performance) with 5-point Likert scales to identify performance gap. The larger number of mean differences between expected performance and current performance of each competency implied the larger performance gap to be improved (or needed). The content of competencies in need analysis questionnaire consisted of 5 domains i.e., dental assisting (AS), health promotion and disease prevention (PP), patient care (PC), management (MN) and NT, each domain had sub-domains, and each sub-domain contained item competencies. The content validity was assessed by 3 experts i.e., dentist, DA, and behavioral researcher. The content validity test of questionnaire was 0.67-1.00. The reliability test was conducted by 30 pilot participants included 15 dentists and 15 DAs before actual distribution. The Cronbach's alpha coefficient was 0.957-0.975. Then researcher sent invited message with fillable form pdf or link google form questionnaire via email or Line application (individuals or groups) to invited dentists and DAs. Then the questionnaire was distributed to dentists and DAs in 5 parts of Thailand i.e., Central, North-eastern, Southern, Northern, and Bangkok.

Ethical consideration

The protocol of the research was approved from Siriraj Institutional Review Board before conducting the research. The approved protocol was MU-MOU 128/2565(IRB1).

Data analysis

Demographic data was analyzed with descriptive statistic, i.e., percentage, frequency, mean, standard deviation (SD). The domains, sub-domains, and items of Thai DAs' competencies were identified for performance gaps. The calculation of performance gap was mean score of the important level of competency (I) subtracted with those of the frequency (D). The larger the value of the performance gap, the higher improvement was needed. The needs for professional development of Thai DAs were defined by $PNI_{modified}$ values. The formular of $PNI_{modified}$ was (I-D)/D. The priority needs were identified by ranking $PNI_{modified}$ values from larger values to lower values.

The summary of methods was shown in Fig. 1.

Results

The results of this research were divided into three parts: demographics of respondents, performance gap analysis of Thai DAs, and NA and priority needs of the performance gaps for Thai DAs.

Demographics of respondents

A total of 764 questionnaires were sent to dentists and DAs in Thailand from which 428 responses were received (respond rate 56%), consisting of 213 dentists and 215 DAs. Most of the respondents were female both dentists (74%) and DAs (93%). Respondents aged around 20-50 years old (88%). Most of dentist and DAs respondents had work experience 6-10 years (20%) and 1-5 years (24%), respectively. Majority of DAs respondents received salary less than 10,000 Baht (48%). 5% of DAs received the highest salary (>45,000 Baht). Most of DAs graduated from grade 9 (38%) and 21% of DAs graduated from 1-year certified dental assistant program. Both dentist and DAs respondents work at Bangkok (14%), Central (24%), Northern (22%), Southern (16%), and North-eastern (24%).

Performance gaps and priority needs

Performance gap and priority needs were calculated and presented into overall, dentists', and DAs' perspectives.

Overall perspectives

The results of NA of performance gaps and priority needs of Thai DAs' competencies from overall perspectives of both dentists and DAs were determined by 3 levels; domain, sub-domain, and competency. The top five of performance gaps and priority needs were showed in Table 1.

Dentists' perspectives

The questionnaires were collected from 213 dentists in various specialties who worked mainly in government hospital (60%), private hospitals/clinics (38%) and a few in universities (2%). The performance gaps and priority needs of Thai DAs' competencies were shown in Table 2.

DAs' perspectives

The questionnaires were collected from 215 DAs who worked mainly in government hospital (61%), private hospitals/clinics (37%) and a few in universities (2%). The performance gaps and priority needs of Thai DAs' competencies were shown in Table 3.

The performance gaps (Fig. 2A) and priority needs (Fig. 2B) of Thai DAs competency domains ranked from the highest to lowest score were summarized in Fig. 2.

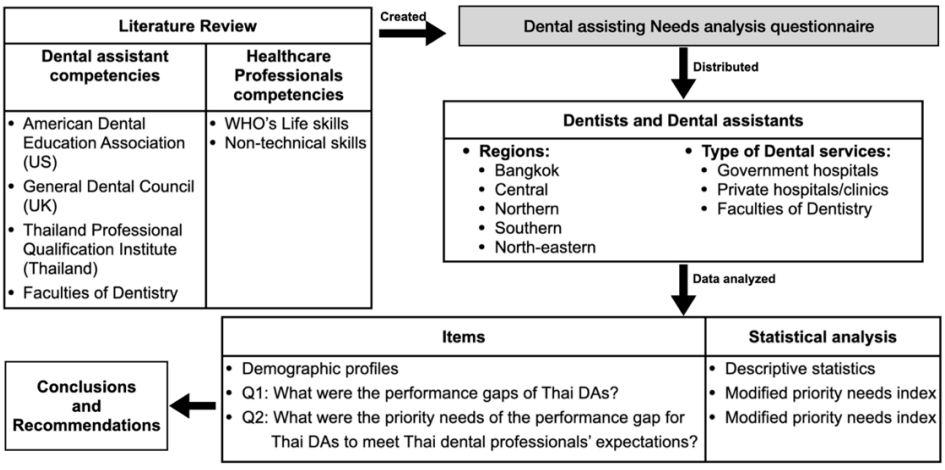

Figure 1. Flow diagram of the research design.

Table 1. Comparison of overall perspectives of top five performance gaps and priority needs of Thai DAs' competencies.

Priority Need	Performance Gap			$PNI_{modified}$	
	Competency	Mean	SD	Competency	Value
	Item competency				
1st	Competently perform basic life support according to current guidelines (AS)	1.325	1.541	Competently perform basic life support according to current guidelines (AS)	0.408
2nd	Perform first aid for common minor accidents and conditions (AS)	1.117	1.131	Support dentists for dental health promotion in the community (PP)	0.306
3rd	Support dentists for dental health promotion in the community (PP)	1.030	1.255	Perform first aid for common minor accidents and conditions (AS)	0.305
4th	Describe the procedures for handling of complaints (MN)	0.972	1.145	Describe the procedures for handling of complaints (MN)	0.271
5th	Explain the important of health promotion for better oral health care (PP)	0.939	1.219	Explain the important of health promotion for better oral health care (PP)	0.267
	Sub-domain				
1st	Completely respond to medical and dental emergencies including basic life support (AS)	1.240	0.954	Completely respond to medical and dental emergencies including basic life support (AS)	0.356
2nd	Support dentists for dental health promotion in community (PP)	1.030	1.255	Support dentists for dental health promotion in community (PP)	0.306
3rd	Explain health promotion and disease prevention in improving oral health (PP)	0.907	1.095	Explain health promotion and disease prevention in improving oral health (PP)	0.251
4th	Effectively manage dental office (MN)	0.819	0.942	Effectively manage dental office (MN)	0.217
5th	Explain the purpose and process of informed consent to patient (AS)	0.799	1.043	Explain the purpose and process of informed consent to patient (AS)	0.202
	Domain				
1st	Health promotion and disease prevention (PP)	0.687	0.800	Health promotion and disease prevention (PP)	0.183
2nd	Management (MN)	0.500	0.639	Management (MN)	0.123
3rd	Patient care (PC)	0.480	0.707	Patient care (PC)	0.121
4th	Dental assisting (AS)	0.478	0.454	Dental assisting (AS)	0.113
5th	Non-technical/soft/life skills (NT)	0.360	0.538	Non-technical/soft/life skills (NT)	0.086

Table 2. Comparison of top five performance gaps and priority needs of Thai DAs' competencies from dentist respondents.

Priority Need	Performance Gap from Dentists			$PNI_{modified}$ from Dentists	
	Competency	Mean	SD	Competency	Value
	Item competency				
1st	Competently perform basic life support according to current guidelines (AS)	2.033	1.195	Competently perform basic life support according to current guidelines (AS)	0.720
2nd	Competently respond to medical emergencies following current best practice guidelines (AS)	1.714	1.123	Competently respond to medical emergencies following current best practice guidelines (AS)	0.534
3rd	Perform first aid for common minor accidents and conditions (AS)	1.573	1.125	Perform first aid for common minor accidents and conditions (AS)	0.486
4th	Monitor the patients to identify any complications (AS)	1.394	1.130	Support dentists for dental health promotion in the community (PP)	0.398
5th	Explain the purpose and process of informed consent to patient (AS)	1.310	1.119	Explain the important of health promotion for better oral health care (PP)	0.393
	Sub-domain				
1st	Completely respond to medical and dental emergencies including basic life support (AS)	1.678	0.910	Completely respond to medical and dental emergencies including basic life support (AS)	0.534
2nd	Explain the purpose and process of informed consent to patient (AS)	1.310	1.119	Support dentists for dental health promotion in community (PP)	0.398
3rd	Support dentists for dental health promotion in community (PP)	1.202	1.318	Explain the purpose and process of informed consent to patient (AS)	0.382
4th	Explain health promotion and disease prevention in improving oral health (PP)	1.164	1.172	Explain health promotion and disease prevention in improving oral health (PP)	0.368
5th	Effectively manage dental office (MN)	1.101	0.949	Accurately record in dental charting according to dentist (AS)	0.346
	Domain				
1st	Health promotion and disease prevention (PP)	0.879	0.842	Health promotion and disease prevention (PP)	0.262
2nd	Dental assisting (AS)	0.700	0.474	Management (MN)	0.183
3rd	Management (MN)	0.690	0.656	Patient care (PC)	0.178
4th	Patient care (PC)	0.637	0.735	Dental assisting (AS)	0.176
5th	Non-technical/soft/life skills (NT)	0.503	0.553	Non-technical/soft/life skills (NT)	0.128

Discussion

From our findings, the majority of DAs in Thailand was female, had work experience of approximately 1-10 years, not trained in certified DA training, worked at government hospitals and had low incomes of less than 10,000 Baht. Low income of DAs is a predictor to low job satisfaction[5-8] and these can cause high stress at work, low emotional well-being, and high intention to leave their job.[6]

The remarkable and highest performance gaps in these results from all respondents was "competently perform basic life support in line with current guidelines" in AS domain which corresponded to dentists' perspective, while DAs' perspective was "monitor the patients to identify any complications and take the necessary action". These raised the notice that the needs from dentists differed from DAs. Dentists focused and are concerned very much in the safety of the patients as demonstrated by the top 3 of Thai DAs' competencies from dentists' perspective. This was because dentists were concerned that DAs are members of the health care team that had to face the crisis together.[9] However, from viewpoint of DAs, they did not as highly value the important of performing basic life support, thus they counted on their role as an assistant, not the leader in medical and dental emergencies and this responsibility belonged to dentists. Nevertheless, there are occasions that DAs can face with medical emergencies such as vasovagal syncope, anaphylactic reaction, orthostatic hypotension, hypoglycemia, seizures, sudden cardiac arrest in dental office.[10,11] Even though these incidences are rare, but they are life-threatening incidence. These finding results are urged for professional development training for Thai DAs to practice and maintain their knowledge and skills in basic life support.

There is an interesting result in non-technical/soft/life skills (NT). On one hand, from this research, performance gaps of NT are lowest from dentists and DAs perspectives. On the other hand, the patients, and their family perspectives required the soft skills such as reliability and responsiveness (willing and prompt to help), which were of highest importance for healthcare professionals, e.g., nurses.[12] These opposite perspectives from healthcare professionals (less concerned) and patients (more concerned) about NT should be markedly considered because many NT are abstract and hard to measure. From literature reviews, NT competencies are important and were recommended from the Dental Associations[13,14] to be taught in DAs training programs.

Table 3. Comparison of top five performance gaps and priority needs of Thai DAs' competencies from DA respondents.

Priority Need	Performance Gap from DAs			$PNI_{modified}$ from DAs	
	Competency	Mean	SD	Competency	Value
	Item competency				
1st	Monitor the patients to identify any complications (AS)	1.214	1.231	Monitor the patients to identify any complications (AS)	0.325
	promotion in the community (PP)			health promotion in the community (PP)	
3rd	Competently respond to medical emergencies following current best practice guidelines (AS)	0.721	1.321	Describe the procedures for handling of complaints (MN)	0.182
4th	Describe the procedures for handling of complaints (MN)	0.712	1.107	Explain the important of health promotion for better oral health care (PP)	0.172
5th	Explain the important of health promotion for better oral health care (PP)	0.688	1.037	Competently perform basic life support according to current guidelines (AS)	0.170
	Sub-domain				
1st	Support dentists for dental health promotion in community (PP)	0.861	1.168	Support dentists for dental health promotion in community (PP)	0.232
2nd	Completely respond to medical and dental emergencies including basic life support (AS)	0.806	0.785	Completely respond to medical and dental emergencies including basic life support (AS)	0.211
3rd	Explain health promotion and disease prevention in improving oral health (PP)	0.651	0.949	Explain health promotion and disease prevention in improving oral health (PP)	0.161
4th	Effectively manage dental office (MN)	0.540	0.848	Effectively manage dental office (MN)	0.132
5th	Describe prevention of common oral diseases and acute oral conditions (AS)	0.473	0.810	Describe prevention of common oral diseases and acute oral conditions (AS)	0.113
	Domain				
1st	Health promotion and disease prevention (PP)	0.498	0.710	Health promotion and disease prevention (PP)	0.120
2nd	Patient care (PC)	0.324	0.642	Patient care (PC)	0.075
3rd	Management (MN)	0.311	0.562	Management (MN)	0.071
4th	Dental assisting (AS)	0.259	0.303	Dental assisting (AS)	0.058
5th	Non-technical/soft/life skills (NT)	0.218	0.484	Non-technical/soft/life skills (NT)	0.049

From our findings, the priority needs of expected performance gaps for Thai DAs, from the 3 highest competencies, were "competently perform basic life support in line with current guidelines", "support dentists for dental health promotion in the community", and "demonstrate knowledge of and ability to perform first aid for common minor accidents and conditions", respectively. The first and third ranks were corresponded to dentists' perspectives. The response to medical and dental emergencies are of high concern due to life threatening potential. In Poland, the prevalence of medical emergencies in dental office was at least once per year and dental hygienists should prepare themselves to help patients. The most common medical situations for dental hygienists in Poland were syncope and mild anaphylactic reaction.[11] The study in Poland supported the findings of this research and recommended dental hygienist to be trained for medical emergencies. Dentists should support DAs to participate in basic life support or cardiopulmonary resuscitation courses every 2 years.[11] Furthermore, the recommendation for dental offices is the basic life support equipment, e.g., automated external defibrillator (AED), life-saving equipment, self-expanding bag resuscitator, oropharyngeal, and supraglottic airway device and oxygen sources, should be available and always be ready to use.

The "support dentists for dental health promotion in the community" competency was important for the community. In Thailand, the distribution of dentists in rural area was low which many patients could not access to dental services. The best way to decrease oral diseases is individuals can take care their own oral health. Previously, dentists should give dental education for patients. Now there are a lot of information which individuals can search from website and youtube. This raises the important of health literacy for individuals. Moreover, WHO promoted health professionals to increase health literacy of patients to increase awareness and capability to gain basic knowledge for taking care themselves through the concept of health literacy,[15] which might be interesting for Thai DAs improvement. In U.S., some individuals have limited to oral health literacy.[16] They may not to access, understand, assess, and apply oral health information for their oral health.[16]

This research showed the priority needs of several competencies for professional development. The situation nowadays is the shortage of DAs in dental services. These situations give a gap for improvement of DA education.

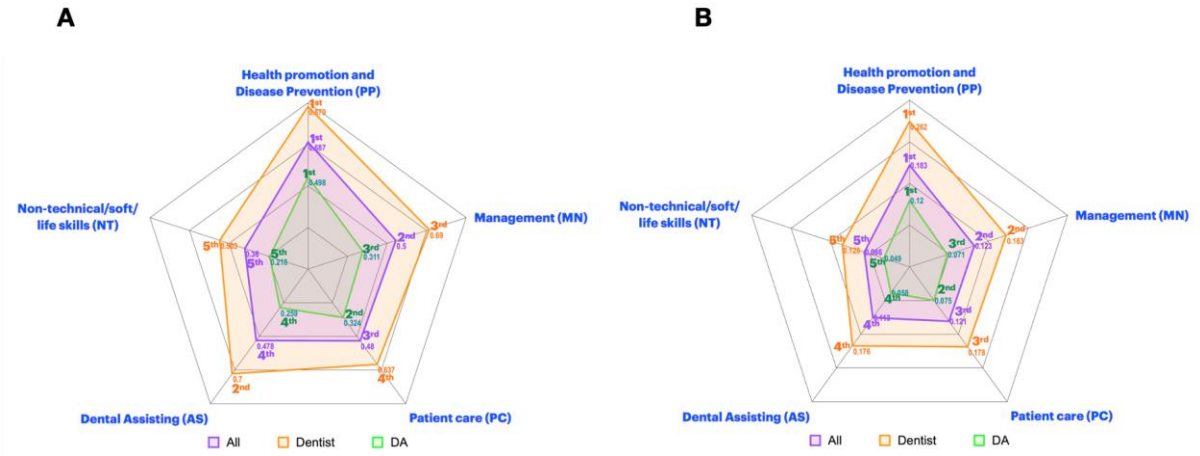

Figure 2. Comparison of performance gap (A) and priority needs (B) of Thai DAs' competency domains from overall, dentists', and DAs' perspective.

Dentists required certified trained DAs to work with.[17] The limitation of Thai DAs to develop themselves are there are a few training programs suit to their needs, cost, and time of training program. The most important competency be concerned is the patient safety that showed, in this results, the highest performance gaps and first priority needs. It is interesting that the opinions of Thai dentists and DAs are different. The international competencies of DAs allowed DAs to do more in operative treatments such as "accurately describe the step of tooth removal" and "explaining different treatment options with the patient, including cost options and the patient's welfare benefits" which Thai dentists might feel that these tasks are the responsibility of dentists not DAs, while US and UK regarded these competencies as requirements for certified DAs. The other reason, in Thailand dental therapists will perform these tasks instead of DAs.

Conclusion

There are performance gaps in dental assisting, health promotion and disease prevention, patient care, management, and non-technical/soft skill/life skill. The top three highest priority needs of performance gap are health promotion and disease prevention, patient care, and management skills. This research demonstrates the performance gaps in professional development of Thai dental assistants, which would be the basis of systematic improvement of dentist assistant training programs.

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Counseling Method Increasing Knowledge, Motivation and Behavior in Mothers of Preschool Children

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ABSTRACT

Background and Objective: Dental caries is mostly suffered by children at preschool age in Indonesia (92.7%), and most of them are untreated. Parents do not have good dental health knowledge, their dental health motivation and behavior are still not support. The aim of this study was to increase the knowledge, motivation and behavior of mothers to care for children's dental health.

Methods: A randomized pretest-posttest control group design was involved 160 mothers of preschool children as samples, divided into two groups: counseling, and control. The study was followed for 2 months. The instrument used were the questionnaires, to determine the knowledge, motivation and behavior of the samples. The data were analyzed with the Paired T-test.

Results: There were significant differences in knowledge, motivation, and behavior before and after treatment (p<0.05) in groups counseling and control, unless there were no significant differences motivation before and after in group control (p>0.05). The counseling group was significantly different compared to the control (p<0.05).

Conclusion: Dental health education with counseling methode can increase preschool mothers' knowledge, motivation, and behavior to care for children's dental health.

Keywords: Behavior, Counseling, Knowledge, Motivation, Mother in preschool

Introduction

The dental caries prevalence in preschool is still high, mostly found without treatment. Dental Health Month 2017 data in Indonesia, shows that at the age of 6 years 74.4% of children with caries (dmf-t 6.29), decay 5.64 per child, and most of them are untreated.[1] The health Basic Research 2018 data shows a prevalence of 92.7% with a DMF-T of 8.43, meaning that every child has 8 to 9 cavities.[2] The government has made efforts to prevent dental and oral diseases for children in the School Dental Health Effort Program,[3] but it didn't work. The method used is considered conventional and lacks parental involvement.

Mothers still do not have good dental health knowledge and have not behaved properly in dental health. Children with toothache, left without treatment.[4] Lack of knowledge is associated with a lack of motivation for parents to carry out dental and oral health checks for their children.[5] Motivation has the greatest influence on children's behavior in maintaining oral hygiene.[6] Parents do not realize that poor child's dental health not only causes toothache and interferes with playing and learning activities. Although there was no significant relationship between dental caries and intelligence, but the prevalence of moderate gingivitis was significantly associated.[7] Severe caries experience was associated with poorer childhood growth and, as such, could be an underinvestigated contributor to stunting.[8]

Mothers always carry cellphones, for social media activities and communicating. Therefore, online counseling methods between doctors and mothers of preschool children are more practical nowadays to provide dental health education, where people are busy with their respective activities, thereby reducing time, cost of transportation and space. Counseling time can be done according to the agreement between the counselor and the counselee. The aim of this study was to increase the knowledge, motivation and behavior of mothers to care for children's dental health.

Materials and Methods

The study determines the effect of dental health education between online counseling with conventional methods on increasing knowledge, motivation, and behavior. The research design used was a randomized pretest-posttest control group design. The measuring instrument used are questionnaires. There are 16 knowledge questionnaires, 11 motivation questionnaires and 16 for behavior. All questionnaires have been declared valid and reliable. The ethical clearance was declared by Udayana University with No.877/Un14.2.2.VII.14/LT/2021.

Sample

There were 160 mothers of preschool of PR. Sarawati Denpasar-Bali involved as samples, aged 30-40 years, have children aged 4-6 years. Mothers have a minimum of high school education and take care of children herself or on a part-time. Then the sample was selected by simple random sampling and divided into two groups: counseling, and control. There were 80 mothers in each group.

Module Book

The module book is a manual that contains about 'children's dental and oral health (for mothers of preschool children)' that given to all samples. This book was previously asked for the opinion of the experts, so that it is suitable as a guide

Counselor

A counselor is a dentist assigned to provide counseling. Involved 10 counselors, a counselor guides 8 mothers. These counselors were previously given training in the form of mastery of dental health education materials for mothers, and counseling technique. The reliability test between counselors was carried out using the Interclass Correlation Coefficient (ICC) test. The reliability value of Alpha is satisfactory (0.997), no-difference between counselors (p = 0.772), and the consistency value between counselors is indicated by the ICC value of 0.997 which means stability between counselors is high. Consultations are given twice a week, for 2 months. The counseling technique used is face-to-face online with video calls.

Data Analysis

Comparative analysis for non-parametric data used Chi square test, while for parametric data with The Paired T-test.

Results

Characteristics of Respondents

Table 1 shows that the education level of mothers in each group is dominated by higher education levels, mothers who work and take care of their own children. There was a significant difference in the characteristics of the level of education (p<0.05). The non-significant differences between the counseling and control groups were seen in the aspects of employment status, and parenting patterns. Although found one different character, in general, the character of these respondents are the same.

Table 1. Demographic characteristic of respondents.

Characteristic		Counseling n	Counseling %	Control n	Control %	*p-value*
Education level	Secondary	6	7.50	16	20.00	0.02
	Advance	74	92.50	64	80.00	
Occupation status	Employee	64	80.00	67	83.75	0.39
	Housewife	16	20.00	13	16.25	
Parenting patterns	Mother herself	55	68.75	58	73.00	0.57
	Part-time	25	31.25	22	27.00	
Age (years)	Range	30-40		30-40		0.07
	Mean ± SD	34.69 ± 3.72		35.49 ± 3.17		

The p-value is generated from the Chi^2 test, except for the age characteristics generated from the Paired T-test

Descriptive Analysis

The Table 2 shows the increasing in the criteria of good knowledge, high motivation and supportive behavior in both groups. The counseling group showed a higher increase than the control group. There were no mothers who had poor knowledge, low motivation and unsupportive behavior towards children's dental health after intervention, both in the counseling and control groups.

Table 2. Knowledge, motivation and behavior criteria before and after intervention.

Variables	Category	Counseling Pre (%)	Counseling Post (%)	Counseling Δ (%)	Control Pre (%)	Control Post (%)	Control Δ (%)
Knowledge	Less	0.00	0.00	0.00	5.00	0.00	-5.00
	Moderate	57.50	15.00	-42.50	52.50	52.50	0.00
	Good	42.50	**85.00**	**42.50**	42.50	47.50	5.00
Motivation	Low	0.00	0.00	0.00	5.00	0.00	-5.00
	Moderate	42.50	5.00	-37.50	42.50	31.25	-11.25
	High	57.50	**95.00**	**42.50**	57.50	68.75	13.33
Behavior	Less support	0.00	0.00	0.00	0.00	0.00	0.00
	Support enough	46.25	17.50	-28.75	53.33	40.00	-13.33
	Support	53.75	**82.50**	28.75	46.67	60.00	13.33

Statistic analysis

The Table 3 shows, there were significant mean differences in all variables in the two groups before and after intervention (p<0.05), except for the motivational variable in the control group (p>0.05), which showed no-significant difference.

Table 3. Variable difference test before and after intervention.

Variables	Intervention	n	Mean ± SD (pre)	Mean ± SD (post)	Differences Mean ± SD	*p-value*
Knowledge	Counseling	80	9.96 ± 2.37	12.50 ± 2.25	2.52 ± 2.13	< 0.001
	Control	80	9.54 ± 2.37	10.51 ± 2.23	0.98 ± 2.04	< 0.001
Motivation	Counseling	80	7.98 ± 1.41	9.74 ± 1.34	1.76 ± 1.86	< 0.001
	Control	80	7.99 ± 1.47	8.09 ± 1.24	0.10 ± 1.31	0.50
Behavior	Counseling	80	10.36 ± 1.69	13.19 ± 2.42	2.82 ± 2.76	< 0.001
	Control	80	10.30 ± 1.93	11.04 ± 1.96	0.74 ± 2.08	< 0.001

Discussion

Relationship of Knowledge, Motivation, and Behavior

Knowledge or cognition is a fundamental domain in shaping one's actions. The knowledge included in the cognitive domain has six levels: knowing, understanding, application, analysis, synthesis, and evaluation.[9] Knowledge and perception will build awareness, providing an impetus to behave.[10] Most of the mothers in this study had good knowledge, high motivation, and supporting factors for dental health behavior in preschool children. The knowledge value of the sample indicates this after treatment, most of which is in the excellent category, except for the control group, likewise, with the value of motivation and behavior. Most of the samples included the category of high motivation and supportive behavior in the three groups. This follows the research of Hamadi et al.[11] who obtained maternal knowledge about good dental caries prevention. In addition, most mothers also have high motivation for child dental care,[12] but this is different from Abdat which shows the behavior of less supportive mothers.[4]

Motivation is an internal condition that produces an individual response to the action, which encourages achieving targets, and stimulates oneself to be interested in certain activities.[13] Individuals are encouraged to take action based on their knowledge and perceptions and behave to achieve the desired goals.[14] There is a relationship between knowledge and motivation on dental health and maintenance behavior. Knowledge and motivation affect dental health maintenance behavior.[15] Attitudes, perceptions, and motivations influence dental hygiene maintenance behavior.[6] In comparison, motivation is an impulse from individuals influenced by their knowledge and perceptions.[15]

Mother's knowledge about children's dental health is related to the behavior of preventing dental caries. Good enough knowledge will significantly impact preventive behavior.[16] Singal et al.[17] showed that mothers with good knowledge about caries' causes (sweet foods and chocolate) showed good ways to take care of their children's teeth daily. Increased knowledge, awareness, and changes in parental behavior are indirectly vital elements for preventing dental disease in preschool children.[18]

Parents' participation in children's dental care is influenced by their knowledge of dental health, attitudes, and motivations toward children's dental care.[5] Pranoto et al.[12] The mothers with high motivation have children with low ECC. The higher the motivation, the lower the child's ECC.

According to Sinaga et al.[18] there is a relationship between knowledge and motivation in homemakers. The better the knowledge about dental caries, the higher the motivation for filling teeth. Zuhriza et al.[19] stated that intrinsic motivation plays a significant role in health behavior, so there is a feeling of guilt in individuals who do not take good care of their teeth. Taking good care of your teeth gives you satisfaction.[20] In addition, keeping the oral cavity as clean as possible gives a pleasant feeling.[19]

Although a person already has his motivation, a person can be motivated by external stimuli. Intrinsic and extrinsic motivation play an important role in determining oral health. The eating habits of parents will affect the eating habits of their children. Parents' motivation to choose positive foods can affect their children's eating patterns and dental status.[20] However, not all suggestions from outside are well received. The respondents that felt pressure from people around them to do dental care.[19] Extrinsically motivated people do not value dental care more often than intrinsically motivated people,[20] so someone returns to the previous behavior.

A mother's knowledge of health care significantly influences children's attitudes and behavior. Children generally cannot maintain oral cavity health, so the parents are responsible for educating them properly.[22]

The impact of GPD and Counseling Methods on Knowledge, Motivation and Behavior.

The values of knowledge, motivation and behavior before and after treatment in the counseling and control groups were statistically significantly different, except for motivation in the control group. In addition, there was also a significant difference in knowledge between the GPD group, and counseling and control. The mean difference in the control value is smaller when compared to counseling. That is, the counseling treatment has an impact on the knowledge, motivation and behavior of the sample. The difference between the counseling with the control group was possible because the counseling group received additional information through face-to-face individually. The counseling samples really took the time to receive information and consult about their child's dental health problems, besides they also received the module. Meanwhile, the control group only receives the module, which may not be read by all its members. There is no-difference in motivation in the control group indicating that better knowledge provides higher motivation. In another study, the same thing was found, that mothers who previously had low and moderate motivation changed to high after receiving dental health education.[23]

There is a relationship between motivation and behavior, the higher the motivation, the better the behavior. Motivation plays a role in the formation of behavior.[24] Tha increasing mother's knowledge will improve her skills in brushing teeth.[25] The lack of knowledge, combined with negative beliefs and behaviors, poor feeding and dental hygiene, and failure of professional dental care, puts children at higher caries risk. Behavioral factors play a role in improving one's health status.[26]

In this study it was found that most of the mothers had good knowledge. This is in accordance with the opinion of Veriza and Riyadi that there is a significant relationship between knowledge and mother's motivation in maintaining dental health.[27] Mothers who have good knowledge, have high motivation to see their dental health. So should it be. However, this opinion contradicts Maulidah et al.[28] that there is no relationship between knowledge about oral health and motivation for dental care. There is no guarantee that people who have good knowledge will be motivated to behave according to their knowledge.

Motivation can be built, someone who has motivation will try to solve the problem until the goal is achieved. The stronger the motivation, the better the behavior in maintaining oral and dental hygiene.[6] Implementing oral hygiene measures no later than the time of eruption of the first primary tooth. Toothbrushing should be performed for children by a parent twice daily, using a soft toothbrush of age-appropriate size.[29] However, in India, almost 65% parents say they will start brushing their child's teeth, after all the primary teeth have grown, the child is about 3 years old.[30]

The counseling method is very suitable to be used to deal with cases of mothers and children who have problems with dental health. The weakness of this research is, because the research was conducted online, it can only be done if the mother has a mobile phone. In addition, because of the mother's busy schedule, counseling sessions are often rescheduled, which makes the counselor less enthusiastic.

Conclusion

Dental health education with counseling increase motivation for mothers of preschool children more than conventional methods. There is a significant difference between the counseling with conventional methods.

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OM-00032

The Development of Glass Ionomer Cement to Improve Fluoride Release Ability

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ABSTRACT

Background and Objective: Fluoride release (F-release) is the most remarkable feature of conventional glass-ionomer-cement (GIC), but it has a "burst effect" F-release pattern. If the high F-release is maintained for an extended period, it will be beneficial in preventing caries. Chitosan has numerous beneficial properties, including F-release control. This study, therefore, aimed to improve F-release of GIC by incorporating chitosan (C) or its nanoparticles.

Methods: Chitosan nanoparticles (CNs) were synthesized from 250-kDa chitosan. Chitosan and CNs were dissolved in 1% acetic acid to obtain a concentration of 0.2 mg/mL. Each chitosan solution was mixed with the liquid phase of GIC at a 10%(v/v) ratio before being combined with the powder part. Eighteen specimens were randomized into 3 groups: GIC, GIC+C and GIC+CNs. The F-release was measured at time interval of 1, 6, 12 hours, 1-7, and 14 days and the cumulative F-release was calculated. The cumulative F-release at day 14 was analyzed by one-way ANOVA with Tukey's post-hoc test (α=0.05).

Results: GIC+CNs (904.85±466.56 μg/cm^2), but not GIC+C (712.72±402.9 μg/cm^2), had significantly higher cumulative F-release at day 14 than GIC (253.49±31.42 μg/cm^2, p<0.05). Similar F-release patterns were seen in the chitosan groups, which produced great amounts of fluoride over 24 hours as opposed to just 6 hours in GIC. The release was then gradually decreased by all groups.

Conclusion: Both types of chitosans increase F-release, particularly CNs, which differed significantly from conventional GIC. The high fluoride levels in the chitosan groups were maintained for a longer period as F-release increased.

Keywords: Chitosan, Dental caries, Fluorides, Glass ionomer cements, Nanoparticles

Introduction

Atraumatic restorative technique (ART) and interim therapeutic restoration (ITR) are commonly used as treatments for high caries-risk patients or when definitive permanent restoration cannot be performed.[1] Conventional glass ionomer cement (GIC) has been a material of choice for both techniques due to its ability to release fluoride, ease of manipulation, and tooth-bond. Although fluoride can inhibit enamel and dentin demineralization *in vitro*, clinical evidence for an anti-caries effect is still questionable.[2] Moreover, when compared to resin composite, the ability in reducing secondary caries of GIC was not different.[3] Furthermore, the GIC's initial burst causes huge amounts of fluoride to be release during the first 24 hours before resting in lower amounts,[4] which may affect the long-term potential to prevent caries.

Chitosan has been used in various fields of dentistry because of antimicrobial property, biocompatibility, and drug-carrying ability.[5] Several studies have added chitosan particles to different types of restorative materials including GIC. With proper amount of chitosan, GIC significantly had better antibacterial property against *Streptococcus mutans* without impairing the mechanical strength.[6,7] Apart from these properties, high fluoride ion released from the material was observed. Not only the typical chitosan particles, but also chitosan nanoparticles that improved the fluoride release of GIC.[8-10] With these advantages of chitosan, we aim to improve GIC property in terms of increase and maintaining the high amount of fluoride released by incorporating chitosan or chitosan nanoparticles.

Materials and Methods

Chitosan nanoparticles synthesis *(VH research group, Department of Chemistry, Faculty of Science, Chulalonglorn University)*

Chitosan nanoparticles were prepared using ionic gelation method from chitosan with 250-kDa molecular weight and ≥ 90% degree of deacetylation (Marine Bio Resources, Samut Sakhon, Thailand).[11] Chitosan (100 mg) was dissolved in 50 mL of 1%v/v acetic acid for 24 hours to get 0.05%w/v chitosan solution. Then, 50 mL of sodium triphosphate (TPP) solution was dropped into the solution at 1 drop per second. The final concentration was 0.434 mg/mL TPP + 1 mg/mL chitosan (molar ratio of TPP: glucosamine = 0.2:1). The particles were rinsed with ethanol and precipitated by centrifugation at 6000 rpm for 15 minutes to remove the excess and vacuum dried. The obtained chitosan powders were kept in a sealed container at room temperature until used.

Sample size calculation

The sample size was calculated based on the mean and standard deviation values of the fluoride release at day 15 between resin-modified GIC (RMGIC) with and without chitosan[12] using the G*Power 3.1.9.4 program with two independent means, power 0.8 and significant level 0.05. The results were 5 specimens per group with 20% compensation, for a total of 6 specimens per group.

Specimen preparation

Fuji IX hand-mixed type (GC Gold Label HS Posterior EXTRA, GC Corp., Tokyo, Japan) was used as a control group in this study. Furthermore, GIC with chitosan (GIC+C) and GIC with chitosan nanoparticles (GIC+CNs) were used as test groups. According to sample size calculation, eighteen specimens (n=6/group) were used in this experiment. To prepare the chitosan solution, 0.2 mg of chitosan or chitosan nanoparticles were dissolved in 1% acetic acid to achieve a concentration of 0.2 mg/mL, which was then used to replace GIC liquid at a 10%(v/v) ratio.[8] The specimens were prepared according to the company's instructions, with a modification in the mixing process. Briefly, GIC powder and liquid, which in the test groups contained a 10%(v/v) chitosan solution, were weighed in ratio 3.4 to 1 g and mixed in an amalgam capsule with a mixing machine (Silamat S6, Ivoclar Vivadent, Austria) for 10 seconds to ensure homogeneously mixing.[13,14] The cement was loaded into a cylindrical metal mold with a diameter of 4 mm and a thickness of 6 mm, then covered with a glass slide to remove excess cement and achieve a smooth surface. The molds were left at room temperature for 6 minutes to allow the cement to set before being examined for fluoride release.

Determination of fluoride release

To determine the fluoride release, each specimen was immersed in 10 mL deionized water (DI) and incubated at 37°C. At the end of each time point (1, 6, 12 hours, 1-7, and 14 days), the specimens were transferred to 10 mL of fresh DI, whereas the remaining DI was used to determine the amount of fluoride released from the specimens using a fluoride ion selective electrode (Versa Star Pro™, Orion™, Thermo Scientific™, USA). The remaining DI was mixed in a 10:1 ratio with TISAB III solution (Orion ionplus®, Thermo Scientific™, USA). The electrode was placed in the mixed solution after being calibrated with 0.1, 1, 10, and 100 ppm fluoride standard solutions (Orion ionplus®, Thermo Scientific™, USA). The fluoride release was measured in ppm and converted to μg/cm² using the equation below.[15] The measurement was carried out in triplicate.

$$\frac{\mu g}{cm^2} = \frac{ppm\ (\frac{\mu g}{mL})}{specimen\ total\ surface\ area\ (cm^2)} \times solution\ volume\ (mL)$$

The amount of cumulative fluoride release at day 14 was the sum of the fluoride releases at each time point from the beginning to the end of the experiment.[15]

Statistical analysis

All statistical tests were analyzed and conducted using SPSS version 28.0 software (IBM, Chicago, IL, USA). One-way ANOVA followed by Tukey's post-hoc test was used to compare cumulative fluoride release at day 14. P-values less than 0.05 were considered statistically significant.

Results

The amount of cumulative fluoride release after 14 days of GIC+CNs (904.85±466.56 μg/cm²) was significantly higher than that of GIC (253.49±31.42 μg/cm², p<0.05), but not of GIC+C (712.72±402.9 μg/cm²) (Fig. 1). Although the cumulative fluoride release of GIC+C was higher than that of GIC, the difference was not statistically significant (Fig. 1).

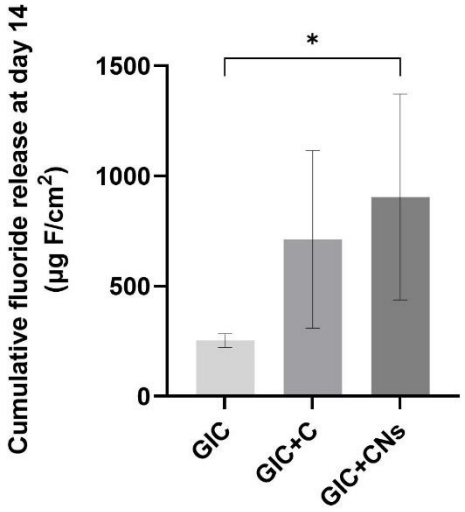

Figure 1. Cumulative fluoride release after 14 days of GIC (control), GIC containing chitosan (GIC+C), and GIC containing chitosan nanoparticles (GIC+CNs). The asterisk (*) represented a statistically significant difference (p<0.05).

Figure 2 depicted the cumulative fluoride released, which the slope represented the rate of fluoride release. The fluoride released pattern of the chitosan groups was similar, with the great fluoride release being detected within the first 24 hours, in contrast to GIC, which was observed at the first 6 hours. After that, the slope was roughly horizontal, indicating that there had been a small amount of fluoride released. It is noteworthy that the amount of fluoride in the chitosan groups was maintained at a high level for a longer period of time than that of the GIC group, which may be due to their higher fluoride release (Fig. 2).

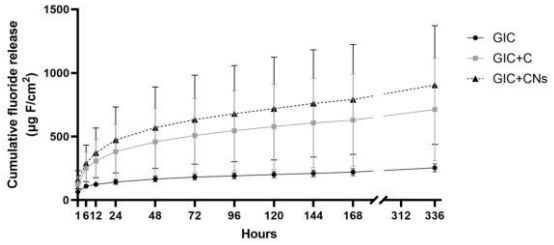

Figure 2. Cumulative fluoride released of GIC (control), GIC containing chitosan (GIC+C), and GIC containing chitosan nanoparticles (GIC+CNs).

Discussion

The aim of this study was to improve GIC property in terms of increase and maintaining the high amount of fluoride released by incorporating 250-kDa chitosan or its nanoparticles (GIC+CNs). Both types of chitosan increased fluoride release, but only GIC and GIC+CNs showed a significant difference. The "burst effect" was still visible in the fluoride release patterns of all groups, but the chitosan groups enhanced fluoride release, causing the high fluoride level to be sustained over a longer period of time.

The amount of fluoride ions released by both chitosan groups was greater than the amount released by the conventional GIC. This phenomenon may be explained by the reaction between chitosan chains and the polyacrylic acid of GIC. Fluoride ions are released and diffuse to the surface of the GIC after the polymeric acid

attacks the fluoroaluminosilicate glass particles during the setting mechanism. When chitosan is added to GIC, the particles bind with the acid, resulting in a more energetic system and faster fluoride release.[8]

The fluoride released from the GIC+CNs group was higher than the GIC control after 14 days, which was consistent with previous studies. Kumar et al. discovered that after 7 days of immersion in distilled water, GIC containing chitosan nanoparticles released significantly more fluoride than the conventional GIC.[9] A recent study also found that chitosan nanoparticles could nearly double the amount of fluoride ion released from various types of GIC over the course of storage.[10]

Even though the GIC+C group released more fluoride, there was no difference with the GIC control. This finding contradicted a previous study, which found that chitosan increased the fluoride released by GIC approximately tenfold after 21 hours.[8] This may be due to the different GIC used which could contribute to different amount of fluoride release because of the material compositions. In addition, the amount of fluoride released from restorative materials depends on several factors such as dimension and storage media.[4] The amount of fluoride released also affected by temperature, as higher temperature accelerates the water diffusion through the cement matrix which resulting in higher fluoride released.[16] Lastly, the specimen dimension, larger surface area exposed in the solution leads to more diffusion area.[17]

The significant difference in cumulative fluoride release between GIC and GIC+CNs but not GIC+C may be explained to the smaller particle size of nanoparticles, which increases the surface area and enhances chemical activity.[18] Since there are more nanoparticles per mass unit than there are larger particles, there is more surface area accessible for chemical interactions between acid and nanoparticles,[19] which results in a higher release of fluoride.

Another expectation from chitosan was the ability to sustain a certain level of fluoride release, in addition to increasing it. The GIC incorporated with chitosan, or its nanoparticles seems to extend the high fluoride level for longer period than conventional GIC, which may be the result of increasing the fluoride release.

The increased and maintained release of fluoride is particularly beneficial in preventing dental caries. In addition, chitosan has an antimicrobial property, and the material must pass some mechanical and physical properties test as well as the required biocompatibility test before it can be used in a clinical setting. Therefore, other than the mechanical and physical properties tested compliance with the International Organization for Standardization (ISO 9917-1) criteria, future studies should also investigate the antimicrobial property of this material.

Conclusion

Chitosan increased the amount of fluoride released from GIC; in particular, chitosan nanoparticles produced significantly more fluoride than conventional GIC. GIC with chitosan or its nanoparticles maintained the high level of fluoride for a longer period of time than conventional GIC, which may due to the increased fluoride release.

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OM-00033

The Immediate Effect of Using Bisdemethoxycurcumin and Potassium Iodide aPDT on *Candida Albicans* Biofilm

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ABSTRACT

Background and Objective: With the continuing emergence of resistant strains in oral candidiasis, current antifungal therapies have proven to be ineffective especially in immunocompromised patients. The present study evaluated the effectiveness of antimicrobial photodynamic therapy (aPDT) using bisdemethoxycurcumin (BDMC) as photosensitizer and potassium iodide (KI) as photocatalyst in the reduction of *Candida albicans* biofilm *in vitro*. The study intended to find the optimum PS concentration and aPDT protocol that can inhibit >3$\log_{10}$ of *C. albicans* biofilm.

Methods: Culturing of *Candida albicans* biofilm (ATCC 10231) was done via an *in vitro* biofilm development assay. Photosensitizer solutions (PS) were prepared using 20/40/80 µM BDMC and 100 mM of KI. Photoirradiation was performed using a dental blue LED light (430 nm, irradiance 500 mW/cm^2) and a fluence of 75 and 150 J/cm^2. The antifungal ability of 14 PS concentrations was evaluated using the colony forming units assay and was measured in $\log_{10}$ CFU/mL/biofilm weight post irradiation at 0 h and 24 h. Shapiro-Wilk test and Kruskal-Wallis H test with Dunn test were used for statistical analysis, with *p*-value<0.05.

Results: Immediately after aPDT irradiation using both 80 µM BDMC and 100 mM KI (fluence 150 J/cm^2), there was complete inhibition of *C. albicans* biofilm with a reduction of 6.01$\log_{10}$.

Conclusion: aPDT with both BDMC as PS and KI as photocatalyst, reaching an optimum PS concentration and light fluence are all requisites in achieving an immediate killing of *C. albicans* biofilm *in vitro*.

Keywords: Antifungal, Bisdemethoxycurcumin, Candida albicans, Photodynamic therapy, Potassium iodide

Proceeding

Introduction

Oral candidiasis is a condition where there is fungal overgrowth in the oral cavity and is commonly seen in the extremes of age: the infants and the elderly. This condition often develop concomitantly with immunosuppression, impaired health status and those who are taking steroids and multiple medications.[1] Antibiotic resistance and resistant strains have continued to emerge and is posing a threat in the effectiveness of the current antifungal therapies especially in heavily immunocompromised patients.[2] Biofilms have been a major concern due to its role in the increasing incidence of antibiotic resistance. Research studies have continuously sought alternative antifungal therapies as solution.

Selective laser therapy such as antimicrobial photodynamic therapy (aPDT) has been emerging as an alternative in treating oral candidiasis.[3,4,5] With the reactive oxygen species generated from the light activation of a photosensitive substance, microbial reduction is demonstrated.[6,7,8] There have been previous aPDT studies that have tested various photosensitizers, concentrations, and irradiation protocol. However, to this date, there is no established aPDT protocol using bisdemethoxycurcumin as PS and potassium iodide as photocatalyst, that can produce ≥3$\log_{10}$ reduction of *C. albicans* biofilm.

Bisdemethoxycurcumin (1E,6E)-1,7-bis(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione) (BDMC) is a natural curcuminoid extracted from *Curcuma Longa* (turmeric). It is a yellow-orange dye substance that exhibits antimicrobial properties. Among the curcuminoids: BDMC as photosensitizer (absorption range of 430-480 nm) tended to result in greater candida inhibition than demethoxycurcumin[9] and was also found to produce more reactive oxygen species and free radicals[10] in short irradiation times.

Several inorganic salts have been previously used as photocatalysts in photodynamic therapy and among these is potassium iodide (KI).[11] Studies have demonstrated that the use of both methylene blue/toluidine blue/nitroindazole and KI as photosensitizers could potentiate greater killing effect.[12,15] Evidence showed that there was a difference of 2-6 logs in microbial reduction[11,14] when KI was added compared to the groups with single photosensitizers alone.

Lasers are commonly used in photodynamic therapy. However, in the present study, the researchers considered the use of a blue LED light which is a basic equipment in dental clinics, not costly as lasers and has a spectrum of 430-480 nm that matches the activation wavelength of the photosensitizers BDMC and KI.

This study explored using BDMC as the photosensitizer and KI as photocatalyst in aPDT to inhibit *C. albicans* biofilm using dental blue LED light (430 nm). The combination of this photosensitizer and photocatalyst with different concentrations and fluence rates in varying post irradiation time points have yet to be tested whether it can inhibit *C. albicans* biofilm *in vitro*.

Materials and Methods

Materials

The main cell line used in this study was a standard *Candida Albicans* strain from ATCC CRM-10231 (American Type Cell Culture Collection, Gaithersburg, Maryland, USA).

The following main reagents were used in the study: Bisdemethoxycurcumin ((1E,6E)-1,7-bis(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione), (Sigma-Aldrich®, Darmstadt, Germany), Potassium Iodide ReagentPlus 99% (Sigma-Aldrich®, Darmstadt, Germany) and Nystatin Oral Suspension (Tystatin) (TOPHARMA Co., LTD, Bangkok, Thailand).

Visible Spectrophotometer (Thermo Scientific GENESYS 20, NY, USA) was used in the study for obtaining the optical density of the cell suspension. Dental blue LED light (Elipar DeepCure-L Curing Light, 3M, Singapore) was the light source used for the aPDT treatment.

Methods

In Vitro Biofilm Development Assay

Culturing of Candida Albicans Biofilm

The ATCC (CRM-10231) cell line, a standard *Candida albicans* isolate from a human with bronchomycosis, was used in this study to test the protocol's antimicrobial effectiveness especially in resistant strains. For the culturing of *C. albicans*, 1 loop of ATCC 10231 was streaked on Sabouraud's Dextrose Agar (SDA) plate and incubated at 37°C for 48 h.[16] Inoculation of 1-2 colonies in Sabouraud's Dextrose Broth (SDB) was done and harvested after 18 h.

This *in vitro* biofilm assay consists of steps that correspond accordingly to the phases of *C. albicans* biofilm formation: (1) adherence, (2) proliferation, (3) maturation and (4) dispersion.[17,18,19]

Cell Density Determination Using a Visible Spectrophotometer

After incubation, an optical density assay was performed using a visible spectrophotometer (Thermo Scientific GENESYS 20, NY, USA) to adjust the concentration of the cell suspension to 10^7 cells/mL (OD = 0.38), with the wavelength set at 520 nm. An optical density of 0.38 is optimal for *C. albicans* biofilm formation.[17] This study made use of 18 x 18 glass cover slips in order to provide a surface for the yeast cells to seed the biofilm for adherence.

1 mL cell suspension was then transferred onto each glass cover slip (placed in 6-well plates) and was incubated for 1.5 hour using an orbital shaker (37°C, 75 rpm) to initiate proliferation of the adhered cells on the glass cover slip.

Colony Forming Units Assay

After incubation, washing of the glass cover slips with phosphate buffer solution (PBS) was done in order to remove yeast cells that are non- and weakly-adhering. It was supplemented with a 2 mL solution of Yeast Nitrogen Base and 50 mM glucose. For further maturation of the biofilm, the 6-well plates with the glass cover slips were incubated for another 48 hours (37°C, 75 rpm). After 48 hours of incubation, the dry weight of the biofilm was recorded (this biofilm dry weight was later measured with the viable cell count to obtain the CFU/mL/biofilm weight).

After photoirradiation of the 48-hour mature biofilm samples (contained on glass cover slips), ultrasonic vibration of these 6-well plates was done to separate the cells. After sonication, the cell solutions were prepared for centrifugation to be plated onto SDA culture plates for drop plating.

Antimicrobial Photodynamic Therapy

Preparation of Photosensitizers

Bisdemethoxycurcumin (BDMC) was mixed with 1% v/v methanol in phosphate buffer solution (PBS) as the diluent. The solution was prepared in an amber colored glass bottle. In order to increase the solubility of the solution, it was heated in a 70°C water bath for 5 minutes. The following final concentrations of BDMC were prepared: 80 µM, 40 µM, 20 µM.

Potassium iodide was diluted with PBS. A final concentration of 100 mM of potassium iodide was used for this study.

Single and mixed solutions of BDMC per concentration and KI were prepared in order to test the effectiveness of both single and double photosensitizers.

To avoid photodegradation and confounding factors such as exposure from natural light, all photosensitizer solutions (PS) were prepared in an amber colored glass bottle. Since all photosensitizer solutions were prepared freshly and used within 1 h, no stock solutions and serial dilutions were necessary.

Nystatin oral suspension 1:100,000 U/mL was used as the positive control and PBS alone was used as the negative control.

Photodynamic Therapy: Photoirradiation Protocol

1 mL of photosensitizer solution (PS) was placed into the 6-well plates containing the glass cover slips (Fig. 1). The entire procedure was performed in a dark room to prevent interference or reaction from any external light source. Pre-irradiation is a necessary step where the samples (or area of treatment in a clinical setting) are allowed to be in contact with the photosensitizer solution prior to photoirradiation. 20 minutes of pre-irradiation time was done for all samples.

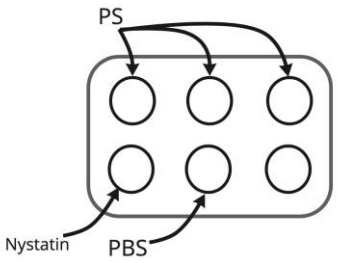


Figure 1. 6-well plate design.

Photoirradiation was performed using a 430 nm blue LED light (3M Elipar Deep Cure-L) in continuous mode (c/w). The area of the light beam is 7.85 cm^2. With a fixed distance of 25 mm from the light source to the target area (bottom of the well plate), the irradiance was adjusted to 500 mW/cm^2 (-10%/+20%). A power meter reader (PM160T-HP, Thorlabs, GmBH) was used to verify and calibrate the power and irradiance. Photoirradiation of the samples was performed with a duration of 2 minutes and 30 seconds and 5 minutes, yielding a an energy density or fluence of 75 J/cm^2 and 150 J/cm^2, respectively. For stability during irradiation, the LED light device was attached to the light apparatus (Fig. 2) which was specifically engineered for the study's use.

Photoirradiation was done using the following photosensitizer concentrations, fluence and corresponding irradiation time (Table 1).

Figure 2. Light apparatus.

Table 1. List of treatment groups.

	IRRADIATION TIME	FLUENCE	PS	PS
1	2 min 30 sec	75 J/cm^2	20 µM BDMC	
2			40 µM BDMC	
3			80 µM BDMC	
4			20 µM BDMC	100 mM KI
5			40 µM BDMC	100 mM KI
6			80 µM BDMC	100 mM KI
7				100 mM KI
8	5 min	150 J/cm^2	20 µM BDMC	
9			40 µM BDMC	
10			80 µM BDMC	
11			20 µM BDMC	100 mM KI
12			40 µM BDMC	100 mM KI
13			80 µM BDMC	100 mM KI
14				100 mM KI
(+)	Nystatin (Positive control)			
(-)	PBS (Negative control)			

Measurement of *Candida Albicans* in Colony Forming Units (CFU)

Drop Plate Method

After photoirradiation and sonication of the samples, a 10-fold serial dilution of the centrifuged pellet (diluted in PBS) was done to obtain 4-5 concentrations: 10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}. Drops of 10 µL of each concentration was plated on SDA culture plates.[20]

Quantitative Analysis of the Viable Cell Count

The number of colonies on the plated concentrations were recorded after a 48-hour incubation (37°C). The median and the interquartile range (IQR) was computed and recorded after an incubation time of 0 h and 24 h post irradiation.

The microbial count or the viable cell count was expressed in $\log_{10}$ CFU/mL/biofilm weight. The dry weight of the biofilm obtained earlier was used to have a more accurate representation and measurement of the remaining viable cells. All experimental groups were performed in triplicate fashion.

Statistical Analysis

Descriptive and inferential statistics were used to obtain the mean, median, IQR, standard deviation and $\log_{10}$ CFU/mL/biofilm weight. The distribution of the data was measured and tested using Shapiro-Wilk test. For the analysis of variance of the data, Kruskal-Wallis H test was performed followed by a post hoc test using Dunn test to determine which among the treatment groups had significant difference in a pairwise manner.

Results

Candida albicans Reduction After aPDT

After aPDT irradiation, all samples were incubated at 0 h and 24 h. Measurements were recorded after 48 h via drop plate method. Nystatin oral suspension (1:100,000 U/mL) was used as the positive control, and phosphate buffer solution (PBS) was used as the negative control. Kruskal-Wallis H test and Dunn test as post hoc was done to assess the significant difference among all the treatment groups (n = 3) with *p*-value<0.05.

After aPDT treatment using different concentrations and combinations of BDMC and KI with 430 nm blue LED light, fluence 75 J/cm^2 and 150 75 J/cm^2 and irradiation time of 2 minutes 30 seconds and 5 minutes, the samples were incubated at 0 h and 24 h. There was no candida inhibition observed in all the treatment groups that were incubated for 24 h and had a similar microbial growth as the negative control (Fig. 3).

At 0 h incubation, when compared to nystatin, all the treatment groups had a similar ability in reducing *C. albicans* biofilm, except for 75 J/cm^2-20 µM BDMC-aPDT with *p*-value<0.05 (Fig. 4a). It could also be noted that the groups with a fluence of 75 J/cm^2 (Fig. 4a), the PS concentrations that contained both BDMC and KI tended to have lower viable cell counts than the single PS groups.

Among all the treatment groups, it was only 150 J/cm^2-80 µM BDMC+KI-aPDT that had a significant difference from the negative control (PBS) with *p*-value<0.05 (Fig. 4b). This concentration also had the greatest reduction in *C. albicans* biofilm out of all the 14 treatment groups.

Using 80 µM of bisdemethoxycurcumin + 100 mM of potassium iodide with 5-minute photoirradiation (fluence = 150 J/cm^2), there was a 6.01$\log_{10}$ reduction of *C. albicans* biofilm which was similar to nystatin (Fig. 5). It was the only concentration that was able to achieve a >3$\log_{10}$ of *C. albicans* biofilm reduction.

Discussion

The treatment groups with two photosensitizers BDMC and KI tended to result in a greater reduction of *C. albicans* biofilm compared to the single photosensitizer groups. Although the difference between the single PS groups and double PS groups was not evident in the groups irradiated with a fluence of 150 J/cm^2, it was only the combination of 80 µM BDMC and 100 mM KI (with fluence 150 J/cm^2) that resulted in a complete inhibition of *C. albicans* biofilm. It could be due to the higher fluence of 150 J/cm^2 that greater microbial reduction could be observed and with the optimum concentration of BDMC; and the addition of KI, complete inhibition was achieved. This finding is as well consistent with the previous aPDT study by Hamblin et al, where there was an additional of 6 logs of killing of microbial species, when KI was added with photofrin.[15]Evidence from previous studies demonstrated that the use of KI with another PS (nitroindazole and MB), an addition of 1-2 logs of killing could be observed.

It could be noted that the high energy density (or fluence) (150 J/cm^2) yielded from the treatment groups aided in the microbial reduction. Liang et al performed antimicrobial photodynamic therapy and tested the difference between using the same photosensitizer and concentration (MB, 50 µg/mL) with varying fluence at 15.2 J/cm^2 and 30.4 J/cm^2. The sterilization effect from the treatment group with the doubled fluence at 75 J/cm^2 was significantly greater than the other treatment group with the lower fluence rate at 15.2 J/cm^2 with *p*-value<0.05.[22] This is consistent with

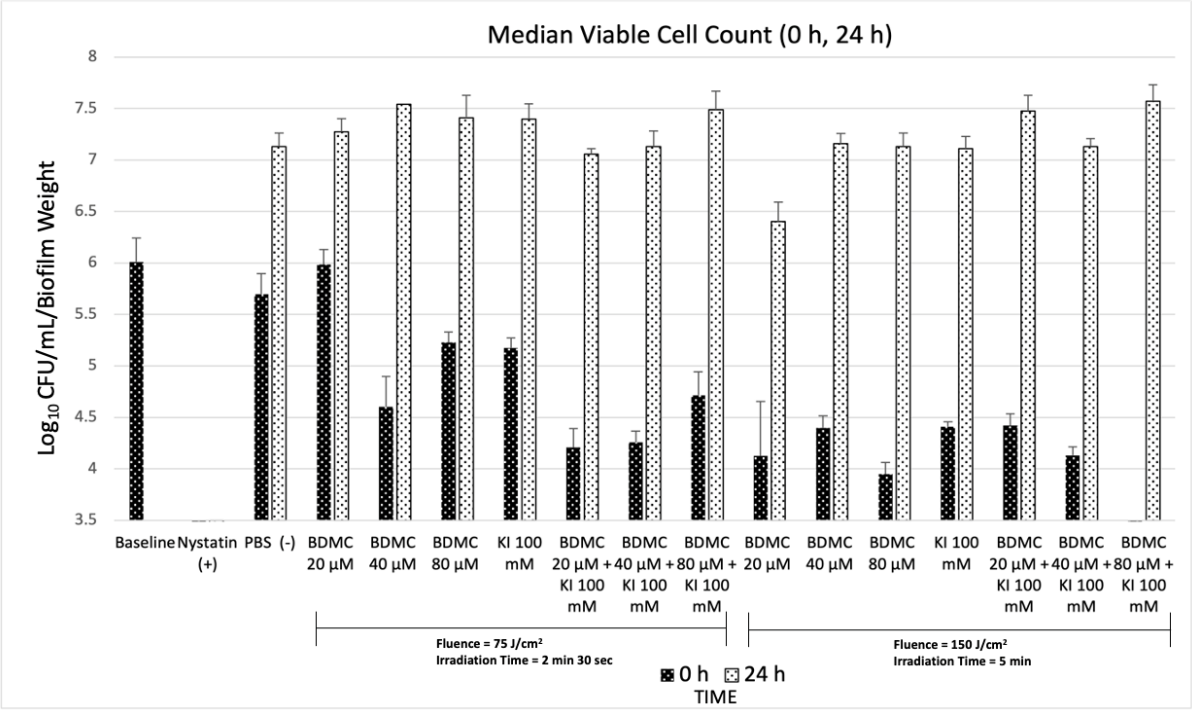
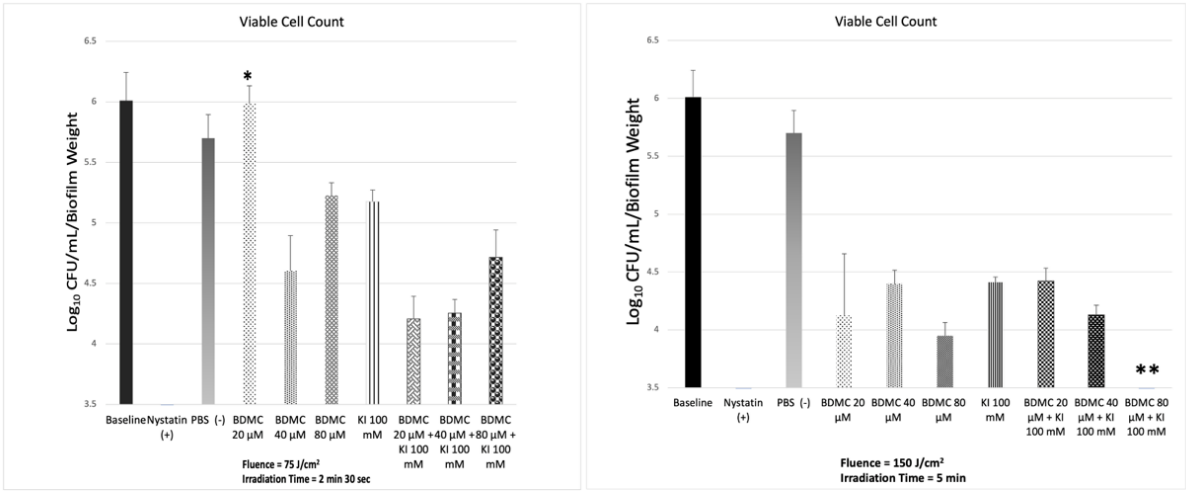


Figure 3. C. albicans viable cell count (median ± IQR) at 0 h and 24 h post-irradiation with 430 nm LED using BDMC 20, 40 and 80 uM alone; and BDMC+100 mM KI, and fluence of 75 or 150 J/cm^2, (n=3).



a b

Figure 4. *C. albicans* viable cell count (median ± IQR) at 0 h after irradiation using 430nm LED for 2 min 30 sec (4a) and 5 min (4b). *Indicates a significant difference from the positive control (nystatin) with *p*-value<0.05, **indicates a significant difference from the negative control (PBS) with *p*-value<0.05.

the current study's findings where the treatment groups with lower fluence rates 75 J/cm^2-80 µM BDMC-aPDT and 75 J/cm^2-20 µM BDMC-aPDT had the least amount of $\log_{10}$ reduction. Among all the 14 treatment groups, these two treatment groups were the only groups that had a significant difference from the positive control (nystatin) with *p*-value<0.05.

The data from the present study suggests that as single photosensitizers in aPDT, BDMC results in a greater log reduction compared to KI. Studies have shown that BDMC generates a high amount reactive oxygen species and free radicals upon irradiation and could be the mechanism behind its antimicrobial activity.[9,10] According to Kazantzis et al, among the curcuminoid derivatives, evidence has shown that BDMC had the greatest potential in cell membrane permeation, enabling a closer interaction with the target site and better intracellular localization.[10,21] Although with the use of this photosensitizer alone, a log reduction greater than 3 could not be obtained, which is the minimum for achieving a clinical therapeutic effect. Since a 6.01 log reduction was obtained with 80 µM BDMC and 100 mM KI (150 J/cm^2) as photosensitizers in aPDT, this particular protocol may have the potential to be an alternative antifungal therapy. Investigations such as cell viability test and cytotoxicity tests are suggested prior to in vivo experiments.

Photodegradation and solubility could be factors that influence the effectiveness of a photosensitizer.[10,21,23] Further studies are recommended to achieve a prolonged photosensitivity when using BDMC as photosensitizer.

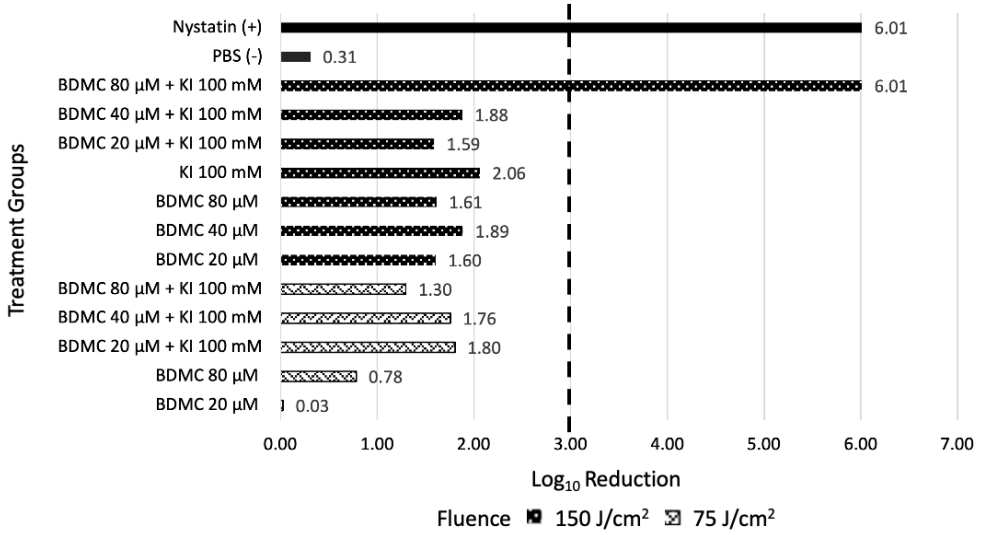


Figure 5. Log_{10} Reduction of all treatment groups. BDMC 80 µM + KI 100 mM (150 J/cm^2) was able to achieve a reduction of 6.01 log_{10} similar to nystatin. >3log_{10} is the minimum log reduction needed to achieve a therapeutic effect.

Conclusion

With the addition of a photocatalyst 100 mM KI to 80 µM BDMC in aPDT (430 nm, fluence 150 J/cm^2), immediate and complete inhibition of *C. albicans* biofilm (with 6.01 log_{10} reduction) *in vitro* could be achieved. The use of a photocatalyst in aPDT can result in a greater antimicrobial activity. Utilizing the optimum concentration of the photosensitizer and fluence are also crucial factors that would greatly influence the microbial reduction.

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