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Original Article **GMSMJ** **Greater Mekong Subregion Medical Journal**

Hand-made Rubber Band Ligater (MUF. - PP Ligater, V1) for Treatment of Hemorrhoids.

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Abstract:

Background: Symptomatic hemorrhoid are common diseases. Most are grade II to III. Rubber ban ligation is the most effective treatment option.

Objective: The aim of the study is to study the efficacy of the hand-made rubber band ligater, PP Ligater, (MUF. - PP Ligater, V1) in treatment grade II to III symptomatic internal hemorrhoid diseases.

Methods: We make the hand-made rubber band ligater from the bubble milk tea straws and then use them to treat grade II to III hemorrhoidal diseases.

Results: Rubber band ligation were undergone with PP-ligater in 116 procedures. No any complications occur during the period of studying.

Conclusions: PP Ligater, (MUF. - PP Ligater, V1) was an acceptable device for grade II to III hemorrhoidal diseases. The hand-made rubber band ligater which made from bubble milk tea straws were used to treat symptomatic grade II to III hemorrhoidal diseases successfully. They made from a cheap material, which easy to make and everyone could make it by oneself.

Keywords: Hemorrhoid, Rubber band ligater, Hand-made device

Introduction

Internal hemorrhoids are common outpatient diseases.[1,2] The non-surgical treatment consist of high fiber diet modifying, some medicines and office base procedures.[3] Blaisdell in 1950s was the first who described a new technique for the ligation of bleeding internal hemorrhoids.[4] The technique was later modified and simplified using rubber bands by Barron in the 1960s.[5] It is now used worldwide because of its simplicity and safety. There are many treatment options but rubber ban ligation are the most effective.[6] One large cases series reported cure rate of 93% and a recurrence rate of 11% after 2 years.[7]

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We choose rubber ban ligation as our treatment of choices for symptomatic internal hemorrhoids. There are many instruments used for hemorrhoid rubber banding. We have made our own rubber band ligater. It is really handmade and made from a very cheap material, bubble milk tea straw.

Objective

Many types of rubber band ligater have some costs and need maintenance. So we make our own rubber band ligaters to use in our outpatients department. They are made from very cheap materials, the bubble milk tea straws, and indeed disposable only one time used. We named it as PP Ligater, (MUF. - PP Ligater, V.1).

How I do it?

1. One pairs of bubble milk tea straw, we should choose them in the difference color. (Figure 1)
2. The outer straw was cut with scissors as shown in figure 2.
3. It was cut through at inferior rim, so it could cover over the inner straw.
4. The outer straw was held with adhesive tape at the cut inferior rim to fit to the inner straw.
5. The inner straw was also cut with scissors as shown in figure 3.
6. The inner straw was cover with the outer one. (Figure 4)
7. A tooth piece insert through the inner straw for straw sliding on each other. (Figure 5)

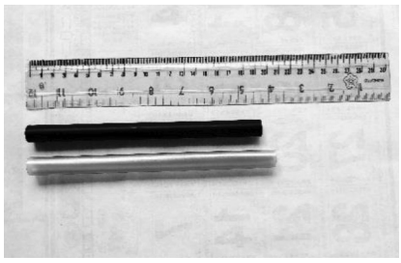

Figure 1

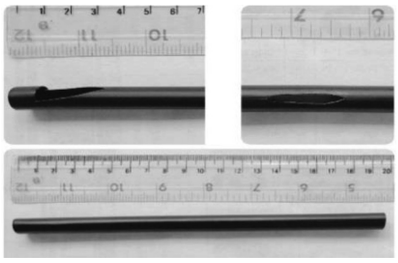

Figure 2

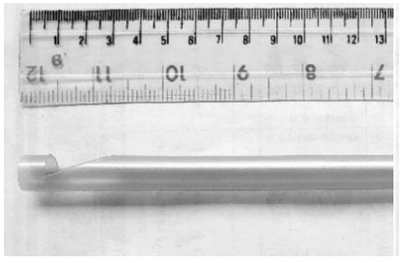

Figure 3

Figure 4

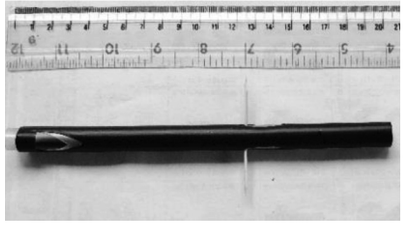

Figure 5

Results

During October 2020 to February 2021, there were 60 hemorrhoid patients. Rubber band ligation were undergone with PP-ligater in 116 procedures as shown in Table 1

Table 1 Characteristics of cases and procedures

Hemorrhoids	Cases	Procedures
Grade II	21	32
Grade III	37	80
Prolapsed rectal mucosa	2	4

The procedures were performed without any sedation or local anesthesia use. The patients could tolerate pain well. Minimal to no bleeding were observed. No any complications occur during the period of studying.

Discussion

Treatment of symptomatic internal hemorrhoidal diseases consisting of adequate fluid and high fiber diet modifying and counseling regarding defecation habits. Medical therapy for hemorrhoids represents a heterogeneous group of treatment options that can be offered with expectations of minimal harm and a decent potential for relief.

Most patients with grade I and II and select patients with grade III internal hemorrhoidal disease who fail medical treatment can be effectively treated with office-based procedures, such as banding, sclerotherapy, and infrared coagulation (IRC). Hemorrhoid banding is typically the most effective option.[8]

The most popular and effective treatment is rubber band ligation (RBL), which has been shown to be superior to sclerotherapy and IRC.[6] The efficacy of RBL in treating grade II and III hemorrhoids was evaluated in a randomized controlled trial (RCT), and after 1 year, 49% of the 176 patients had recurrent hemorrhoidal symptoms, of which the majority were treated with repeat RBL, 32% of the cohort required additional procedures and more than half of which were repeat RBL.[9]

We choose rubber ban ligation as our treatment of choices for symptomatic hemorrhoids. There are many instruments used for hemorrhoid rubber banding. Many types of rubber band ligater have some costs and need maintenance. So, we make our own rubber band ligaters to use in our outpatient department. They are made from very cheap materials, the bubble milk tea straws, and indeed disposable only one time used.

At the Medical Center Hospital (MCH-MFU), there are more than 200 cases of internal hemorrhoids a year. Most of them are classified as grade II to III.

Conclusions

PP Ligater, (MUF. - PP Ligater, V1) was an acceptable device for grade II to III hemorrhoidal diseases. It made from a cheap material. It was easy to make, so everyone could make it by oneself.

Acknowledgement

The author would like to thank to the patients and physicians, participants of this study, for their permission and cooperation. We would also like to thank Roger Timothy Callaghan, MB ChB for his careful reading of our research work and his suggestion for English usage. We wish to express our special thanks to all the staff in Medical Center Hospital, Mae Fah Luang University for their precious assistance, and also giving permission for gathering information.

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A Review of Two-year 2019-2020 Blood and Blood Component Used in Medical Center Hospital of Mae Fah Luang University, Chiang Rai, Thailand.

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Abstract:

Background: Medical Center Hospital of Mae Fah Luang University (MCH-MFU) was opened in December, 2018. It consists with 400 beds but currently initial operation is only 60 beds in 2 general wards and critical care unit. Blood and blood components usage review is a tool for quality monitoring and efficient management.

Objective: The study aimed to know utilization of blood and blood component in MCH-MFU during year 2019 and 2020. The information would guide the executive for the development of the hospital.

Methods: The primary data was collected and retrospectively analyzed. Data between year 2019 and 2020 was compared to see the increment of utilization.

Results: The proportion of blood groups showed that group B and O red blood cell components were mostly used. Increment of blood use was around 1- 2%.

Conclusion: Blood and blood components used in MCH-MFU during 2019-2020 were still low due to the hospital is not yet fully operating. The increment could demonstrate the increasing rate of blood component usage which supported estimation of future blood requirement. Cross match to transfusion ratio reflected appropriateness and improvement of clinical blood use.

Keywords: Blood and blood component, Medical Center Hospital, Mae Fah Luang University

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Introduction

Blood is a resource of medical treatment for the patients with clinical conditions indicated. Medical Center Hospital of Mae Fah Luang University (MCH-MFU) has been founded since 2018, only about 2 years-service, with a vision to be a leader medical center hospital for the people in Chiang Rai province and upper northern part of Thailand as well as for the Greater Mekong Subregion (GMS) countries. The infrastructures and facilities are consisting of 400 beds, operating rooms, labor rooms general laboratory including blood bank lab, outpatient departments and emergency room, x-ray department etc. MCH-MFU aims to be a medical school hospital with several centers of excellence (COE). Currently major constraints are the limitation on recruitment of medical specialists and staff as well as the limitation of patients' accessibility. As the results, the number of patients, both out-patient and in-patient, is not large and only 60 beds for in-patient wards and critical care unit are operating. However, it is expected to grow up rapidly which will affect blood supply management.

A two-year 2019-2020 review used primary data of blood and blood components usage in MCH-MFU since March 2019 to December 2020 to demonstrate number of blood component requested and delivered for transfusion as well as cross match to transfusion ratio. General information in blood groups (A, B, O, AB) used is also prescribed. This review will help understanding blood utilization pattern, leading to future preparedness of blood resource and management as well as improve clinical and academic aspects on appropriate use of blood.

Material and Methods

Primary data from Blood bank division, Pathology section during 2019-2020 was collected and retrospectively analyzed. Packed red cell (PRC), leukocyte reduced packed red cell (LPRC) and fresh frozen plasma (FFP) were 3 blood components (BC) to be reviewed although platelet component (PLT) was also clinically used. Total numbers of BC requested in 2019 and 2020 were showed. The BC delivered for transfusion in 2019 and 2020 were comparably demonstrated in monthly basis. Cross match to transfusion ratio (C/T), blood groups and trends were also observed. This article is limited to deeply review of clinical conditions related to blood transfusion.

Results

In 2019 during 10 months period from March to December, the total number of BC requested were 140 units of PRC, 158 units of LPRC and 25 units of FFP. In 2020, from January to December, blood requirement was gradually increased, the total number of BC requested were 252 units of PRC, 349 units of LPRC and 80 units of FFP.

In 2019 PRC were delivered for transfusion average 14 units per month with the highest number of 25 units in September and November while the lowest is 1 unit in October. LPRC were delivered for transfusion average 15.8 units per month with the highest number of 33 units in July and the lowest was 2 units in April. FFP were delivered for transfusion average 2.5 units per month with the highest number of 6 units in July and there was no FFP transfusion in March, April, May and November 2019.

In 2020 PRC were delivered for transfusion average 21 units per month with the highest number of 45 units in January and no PRC transfusion in August. LPRC were delivered for transfusion average 29.08 units per month with the highest number of 46 units in August and September and the lowest is 11 units in January. FFP were delivered for transfusion average 6.67 units per month with the highest number of

18 units in January and there was no FFP transfusion in April.

Increment rate of PRC used was 1.5% while LPRC was 1.84 and FFP was 2.66%. Comparison of average BC delivered for transfusion during 2019 and 2020 in monthly basis and rate of increment were summarized in Table 1.

Total number for each type of BC requested and delivered for transfusion were calculated to show cross match to transfusion ratio (C/T). In 2019 C/T of PRC, LPRC and FFP were 2.06, 1.98 and 1.19 respectively. The appropriate C/T should not above 2. The fluctuation of PRC C/T above 2 occurred in April = 10, November = 3.1 and December = 2.2, while LPRC C/T in May = 3.0, July = 2.5, October = 2.4 and December = 2.5. In 2020 all BC C/T were below 2 in average. C/T of PRC, LPRC and FFP were 1.79, 1.65 and 1.00 respectively. Nonetheless, the fluctuation of C/T ratio above 2 was also observed and summarized in Table 2.

The proportion of blood groups A, B, O and AB transfused were also demonstrated. In 2019, for PRC group B was highest used at 44.12% of other blood groups, followed by group O at 30.88%, which the pattern was similar to LPRC. In 2020, PRC group O was highest used at 52.48% as well as LPRC group O was highest used at 49.06%. FFP group O was mostly used in both 2019 and 2020. The summarized data are in Table 3 and Figure 1.

Table 1 Comparison of the average BC delivered for transfusion per month in 2019 and 2020 with rate of increment

	2019 Average unit/month [highest, lowest]	**2020** Average unit/month [highest, lowest]	**Rate of Increment%**
PRC	14 [25, 1]	21 [45, 0]	1.5%
LPRC	15.8 [33, 2]	29.08 [46, 11]	1.84%
FFP	2.5 [6, 0]	6.67 [18, 0]	2.66%

Table 2 Cross match to transfusion ratio in 2019 and 2020

	2019		**2020**	
	Average C/T	**C/T ≥ 2 by month**	**Average C/T**	**C/T ≥ 2 by month**
PRC	2.06	Apr =10, Nov = 3.1 Dec = 2.2	1.79	Jun = 3, Sep = 2.6
LPRC	1.98	May = 3.0, July = 2.5, Oct = 2.4, Dec = 2.5	1.65	Apr = 2.1, May = 4.3
FFP	1.19		1.00	

Table 3 Percentage of BC delivered for transfusion by blood groups (A, B, O, AB) in 2019 and 2020

BC transfused by blood group		2019 (%)	2020 (%)
PRC	**A**	20.59	15.60
	B	**44.12**	29.08
	O	30.88	**52.48**
	AB	4.41	2.84
LPRC	**A**	22.50	25.94
	B	**37.50**	18.40
	O	33.75	**49.06**
	AB	6.25	6.60
FFP	**A**	42.86	5.00
	B	19.05	26.25
	O	**47.62**	**68.75**
	AB	9.52	0.00

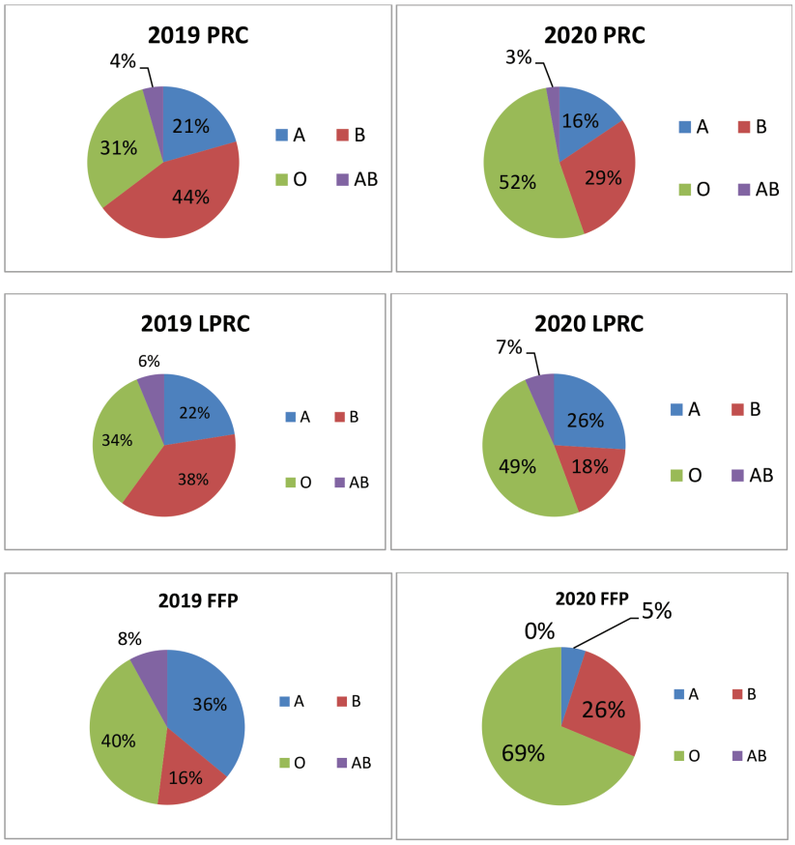

Figure 1 Proportion of blood group (A, B, O, AB) of each BC delivered for transfusion in 2019 and 2020

Discussion

The importance of blood products is further emphasized by inclusion of whole blood, red blood cell, platelets and FFP in the World Health Organization Model List of Essential Medicine[1]. Blood establishments (BEs) including blood center, blood service organization and hospital blood bank have to manage blood services effectively in terms of safe, sufficient and quality-assured. WHO has published the Action framework to advance universal access to safe, effective and quality-assured blood products 2020-2023[2] which includes strategies that BEs should follow in order to achieve the purpose of adequate and safe blood supply. At the level of hospital blood bank, blood utilization review is one of the quality tools for approaching blood utilization auditing[3]. This review has not yet been reached to determine the clinical appropriateness of blood use, but will get statistic data for future preparedness of blood supply and observation of clinical trends for blood use during 2019-2020 in MCH-MFU.

Estimation of blood requirement and preparedness

According to the number of patients was not large, blood was still adequate for requirement. By calculating the increment rate of blood used in 2020, estimate units of PRC, LPRC and FFP requirement in 2021 are 256, 355 and 82 units respectively, which are not significantly increased compared to the previous year. There was no global standard for estimating countries needs for blood/blood products, and estimates have to be made for each country and each region. In February 2010, from the forum of WHO Experts' Consultation on Estimation of Blood Requirements set up at WHO-HQ, Geneva, the expert presentation proposed blood requirement at 10-30 units per bed per year (Super-specialty - 30, Specialty - 20, General -10). In case of MCH-MFU full operation at 400 beds in the level of super-specialty COE, the estimation of blood requirement should be 8,000-12,000 units of red cell component per year.

Cross match to transfusion ratio (C/T)

C/T is one of the tools to observe appropriateness of clinical blood ordering. C/T above 2 should consider over requested from clinicians. Continuing medical education on appropriate use of blood and some other hospital strategies, such as "single unit transfusion policy" and Maximum surgical blood order schedules (MSBOS) should be considered for implementation. High C/T also affects blood stock management in blood bank. The cross matched units may be hold for many days and may not circulate for the other cases adequately; stock number will increase and may lead to expire.

In 2019, C/T for PRC was 2.06 with very high C/T found in several months, but in 2020, this review showed C/T for all BC below 2 and less occurrence of high fluctuation. This satisfactory improvement might result from clinicians' operative experience, appropriate blood use recognition as well as trust in blood ordering system and blood bank management.

Types of blood component

Due to the number of patients was not large and not wide variety of clinical conditions, platelet blood component (PLT) was not often used currently, therefore PLT was not analyzed in this 2-year review.

Shifting usage of PRC to LPRC was found. LPRC is a BC which some of white blood cells (WBC) are removed into buffy-coat layer by centrifugation during the process of preparation in order to reduce complications from WBC transfusion. However, the better way to remove WBC should be filtration, resulting BC as leukocyte depleted (LD), which blood bank of MCH-MFU plans to implement soon.

Blood group mostly used

The majority blood group of Thais is group O, follows by B while blood group A and AB are lesser. In general situation blood group B and O are adequately supplied and sometime over stock of group B, but on the other hand, when blood supply is short, blood group A is usually affected first.

The review showed that in 2019 blood group B transfusion was dominated while in 2020 was blood group O. This simply due to a hematologic malignancy patient with blood transfusion dependent was group B, who received chemotherapy and blood till expired by 2019, then a new case was group O, enrolled in 2020.

Conclusion

Blood and blood components used in MCH-MFU during 2019-2020 were still low due to the hospital is not yet fully operating. Increment of blood use was around 1- 2%. Estimation of future blood requirement depends upon expediting clinical services to its full potential. C/T indicated improvement of clinical requirement that below 2. LPRC usage was increased over PRC. Blood utilization review, patient blood management and hemovigilance are interesting programs which should be considered to implement in the COE hospital as well.

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Review Article **GMSMJ** **Greater Mekong Subregion Medical Journal**

The Presence of Monoterpenes in *Rhynchanthus longiflorus* Hook.f. Confirms the Value of Its Use in Akha Folk Medicine

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Abstract:

Background: *Rhynchanthus longiflorus* Hook.f. is an endangered species and is part of the ginger family. A decoction of its rhizomes is mentioned in Akha folk medicine for the treatment of convulsions.

Objective: With no prior studies performed, it was reasonable to analyze the chemical components of the rhizomes responsible for the odor, taste and perhaps medicinal claims for the plant.

Methods: Rhizomes of *Rhynchanthus longiflorus* Hook.f. were bought from local markets in Chiang Rai, Thailand. They were subjected to hydrodistillation to obtain the essential oil. Analysis of the oil was done by GC-MS.

Results: Fifteen monoterpenes were identified from the essential oil. The most abundant compounds were 1, 8-Cineol (70.705 %), beta-Pinene (7.008%), alpha-Pinene (4.763%) and Sabinene (3.484%) which comprised 85.96 % of the oil. These monoterpenes have been reported to reduce mortality and attenuate seizures in animal models

Conclusion: The essential oil from *Rhynchanthus longiflorus* Hook.f. rhizomes contains monoterpene compounds whose anticonvulsant effects could be the basis for the use of this plant in Akha folk medicine.

Key words: Akha, *Rhynchanthus longiflorus* Hook.f., Monoterpene

Introduction

There are six major tribes or minorities living in the remote hills of northern Thailand. They are the Akha, Mhong, Karen, Lahu, Lisu and Mien. Folk medicine, in which medicinal plants play a major role, has been popularly used by these tribes in the maintenance of health as well as in the prevention, diagnosis, and treatment of physical and mental illness[1,2]. Among the medicinal plants used by these tribal people, *Rhynchanthus longiflorus* Hook.f.,

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an endangered species in the ginger family, is occasionally mentioned. The plant is an epiphytic, perennial rhizomatous herb which can be easily characterized by a profound inflorescence that looks like a bird[3]. Only in some Akha villages, the evidence for its medicinal use was narrated by folk healers, who said that the decoction of the plant's rhizomes could be used to treat convulsions[4].

In May 2015, we found rhizomes of a plant resembling *Rhynchanthus longiflorus* Hook.f. (Figure 1) being sold in the walking street market of Mae Chan district, Chiang Rai province, Thailand. The vendor referred to these rhizomes by an Akha word that means "cliff ginger" and told us that he bought them from the Kentung market in Myanmar. The rhizomes were 4.5–5 x 2.5–3 cm, ovate, faintly aromatic with membranous scale leaves, arranged in a single linear row. There was no inflorescence or other recognizable plant parts, essential for authentication. Therefore, to ascertain that these rhizomes belonged to *Rhynchanthus longiflorus* Hook.f., we cultivated them until their inflorescences appeared.

Convulsion is a neurological disorder which commonly controlled by the anticonvulsants. However, some patients have been experienced uncontrolled seizures and serious side effects due to the drugs[5]. Therefore, finding of new candidate compound with high efficacy and good safety profile would be a valuable strategy for the convulsion control. The rhizome of *Rhynchanthus longiflorus* Hook.f has been previously mentioned for its traditionally use as anticonvulsants. However, there is a lack of robust evidence for the medicinal claims. To fill this gap, we therefore analyzed the chemical components of the rhizomes responsible for the odor, taste, and medicinal claims of the plant.

Materials and Methods

Plant material

Approximately 10 kg. of the rhizomes were bought from the walking street market of Mae Chan district, Chiang Rai province, Thailand in May, 2015. Some of the rhizomes were cultivated in plastic baskets, containing coconut husk chips and pumice, until the inflorescence emerged from the leafy shoot six months later. The whole plant including its inflorescent was assigned as CR-019 and sent to the Queen Sirikit Botanical garden for identification.

Preparation of the essential oil

Coarsely chopped fresh rhizomes (6567.26 g) were hydrodistilled in a Clevenger apparatus After extraction, the organic layer (essential oil) was separated from water by decantation and then desiccated by slow percolation over anhydrous sodium sulphate (Na_2SO_4) in a simple filtration system. The essential oil was stored in amber color vials in cool and dark conditions awaiting further analysis.

Analysis of chemical components by GC-MS

Phytochemical analysis of the essential oil was accomplished using GC-MS analysis. The system comprised a gas chromatograph (GC 7890 Agilent Technologies Co., Ltd., USA) and a mass selective detector (Agilent HP 5973) using a DB-5MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness) with electron ionization (70 eV) and an ion source operated at a temperature of 230°C. The GC oven temperature was set as follows: commence at 50°C and held for 5 min, then slowly raised (1°C/min) to 200°C, and then to final temperature, 250°C (5°C/min) which was maintained for 10 min. The temperature of the injector was held at 250°C; the flow rate of the helium (carrier gas) was maintained at

1.5 mL/min; 1:25 split ratio. About 1 µl of the essential oil was injected into the GC-MS using a micro syringe and the scanning was done for 45 min. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained, which was then processed by computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the retention time (RT). The chemical constituents of the extract were identified by comparing the relative retention times and mass spectra with the data in the NIST05a.L

Results

Plant material

Approximately six months after cultivation of the rhizomes, a beautiful decorative inflorescence emerged from each leafy shoot. The leaves were sessile or shortly petiolate with oblong-lanceolate or elliptic-oblong leaf blades. The inflorescence, 9–10 cm, was an unbranched spike with a short peduncle and a bright red axis slightly curved upwards with 5–6 flowers, all directed upwards as shown in Figure 1. The plant sample (CR-019) was identified by Dr. Jarun Maknoi, Queen Sirikit Botanical garden, Thailand as *Rhynchanthus longiflorus* Hook.f. and the herbarium number, QBG. No. 85757, was assigned to the specimen.

Figure 1 Rhizomes, leafy shoot and inflorescence of *Rhynchanthus longiflorus* Hook.f

Preparation of the essential oil

The chopped rhizomes had an aromatic camphoraceous odor with a pungent, spicy, and cooling taste. From 6567.26 g of the fresh rhizome, 20.0 ml of the essential was obtained. The essential oil yield was thus 0.304 % (w/w).

Analysis of chemical components by GC-MS

Sixteen peaks were distinguished by their retention times. One compound at the retention time of 12.506 min could not be identified. However, the content of this unknown compound was only 0.581% and so could be assumed as negligible. The most abundant monoterpenes from the 15 identified compounds found in the essential oil were 1,8-Cineol (70.705 %), beta-Pinene (7.008%), alpha-Pinene (4.763%) and Sabinene (3.484%) which comprised 85.96 % of the oil (Table 1).

Table 1 Essential oil composition (%) of *Rhynchanthus longifolius* rhisomes

Compounds	Retention time (min)	Content (%)
alpha-Pinene	7.762	4.763
Sabinene	8.740	3.484
beta-Pinene	8.804	7.008
beta-Myrcene	9.142	0.884
3-Carene	9.547	0.538
1, 8-Cineol	10.077	70.705
beta-Ocimene	10.337	0.644
gamma-Terpinene	10.548	0.602
beta-Linalool	11.318	2.515
Unknown	12.506	0.581
4-Terpineol	12.682	1.819
alpha-Terpineol	12.899	2.630
2-Carene	15.226	0.967
trans-Methyl isoeugenol	15.937	1.485
beta-Patchoulene	18.885	0.356
beta-Eudesmol	19.110	1.019

Discussion

The genus Rhynchanthus is represented by six species distributed in Myanmar, India and South China[6]. The Akha tribe, who are aware of the medicinal use of *Rhynchanthus longiflorus* Hook.f. originated in South China but migrated into Myanmar and Thailand around the middle of the nineteenth century[7]. In the present study, we analyzed the chemical components of *Rhynchanthus longiflorus* Hook.f responsible for the odor, taste, and medicinal claims. The Essential oils in which mono-terpenes are the major components of the plant. They have been shown to exert anticonvulsant activity. In 2009, Oliveira et al. reported that the essential oil of Ocimum basilicum increased the latency for development of convulsions in pentylenetetrazol-induced convulsions in mice. The principal components of the oil were 1.8-cineole, linalool, and geraniol, which comprised 92.9% of the oil[8]. Again in 2016, Masoumi-Ardakani et al. demonstrated the anticonvulsant activity of the essential oil of Elettaria cardamomum, an aromatic spice (cardamom) native to the humid Asian areas, in pentylentetrazole and maximal electroshock models in mice. The major compounds in the essential oil were 1,8-cineole (45.6 %), α-terpinyl acetate (33.7 %), sabinene (3.8 %), 4-terpinen-4-ol (2.4 %), and myrcene (2.2 %), respectively[9].

Monoterpenes are characterized by having two isoprene units, each with five carbons (C5) in the structure[10]. One of the key properties of monoterpenes, in the context of epilepsy, is their ability to cross the blood-brain barrier (BBB). Drug candidates likely to cross the BBB are usually of small

size (<400 Da) and high lipid solubility[11]. Virtually all the monoterpenes found in the essential oil of *Rhynchanthus longiflorus* Hook.f. meet these criteria. Monoterpenes also show anti-inflammatory[12] and antioxidant activities[13,14]. These proprieties make them interesting molecules for protection against neuro-inflammation and redox disequilibrium, which have been observed in the neurological disorders occurring in epilepsy[15-17].

Of all the monoterpenes found in this essential oil, 1,8-Cineole, alpha-Pinene, beta-Pinene, Alpha-Terpineol, beta-Linalool and beta-Myrcene, have been shown to reduce mortality and attenuate seizures in animal models[18-23]. We found no reports of anticonvulsant effect of Sabinene but the oil from the dried seed kernel of nutmeg (*Myristica fragrans* Houtt), in which Sabinene was the major active constituent, was demonstrated to exhibit anticonvulsive action[24]. Therefore, it is reasonable to postulate that the presence of these monoterpenes in the essential oil of *Rhynchanthus longiflorus* Hook.f. was responsible for the anticonvulsant effect of this medicinal plant.

Conclusion

In conclusion, the essential oil from *Rhynchanthus longiflorus* Hook.f. rhizomes contains monoterpene compounds whose anticonvulsant effect could be the basis for the use of this plant in Akha folk medicine.

Acknowledgments

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Conflicts of Interest

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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How to Improve Learning Achievement of Medical Students Regarding Interpretation of Hematology Slides.

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Abstract:

Background: It is essential that Thai medical students learn how to interpret abnormalities of red blood cells, white blood cells and platelets from peripheral blood smears. Following this they need collate this information and make a correct diagnosis of the common hematologic diseases found in Thailand. Because of time constraints, high student to teacher ratios, there being relatively few hematology and clinical pathology specialty clinical teachers, there is a particular need to trial various teaching methods, in order to improve learning outcomes, in relation to the ability of the students to correctly interpret hematology slides.

Objective: The author would like to know the efficiency of different learning methods by using an immediate answer feedback following each quiz of peripheral blood hematology slides. With this method, the author would like to measure the progress of the medical students by comparing scores from the tests.

Methods: Before learning, the medical teacher gave the third year medical students a paper on how to interpret a peripheral blood smear, for self-study. To promote active learning, 2 days after self-study, the medical teacher presented the first session, called **demonstration slides** (14 diseases or conditions) as seen using a light microscope. This was conducted in an OSCE-examination style, 5 minutes per station, in order to ensure that all students examined the slides during the session. There was no formal marking of this session. Immediately after the demonstration test, the teacher gave the answers and pictures from the light microscope online to all students, via Google classroom. In the afternoon of the same day, the teacher conducted a second **quiz,** with slides of 14 different diseases and collected the scores from each student. Again, following completion the answers were revealed. The students were then able to check their results via Google classroom or LINE social media. One week later, at the time of **final examination,** slides of 23 diseases (some diseases were repeated but different questions or from different patients) were presented, the teacher then compared the scores from this last test to the second test and the answer was again sent to all students via the same channels. One way ANOVA test was used for

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analyzing mean differences between tests with p-value < 0.05 at 95% confidential interval (CI) was considered to be significant.

Results: There were 32 medical students in the class. Thirty of 32 students (93.8%) showed improved scores from the first to the second test. Thirty of 32 students (93.8%) had improvement from the second test to the final examination. Mean points ± S.D. scores from the first test were 2.33 ± 0.97 compared to 4.33 ± 0.97 in the second test and 6.07 ± 0.81 in the final examination. All 32 students achieved significantly improved scores ($p<0.001$) from the first to the final test, confirming increased learning achievement.

Conclusion: The examination-based teaching was shown to be an effective way to improve the learning of hematology slides. This teaching concept was stimulating, consumed less time, was cost-effective, had no requirement for high technology equipment and the students were able to learn about many diseases at the same time. The immediate feedback of answers, linked to pictures from the light microscope, was a key factor leading to this success. However, the retention of knowledge needs either repetitive examination or promotion of active learning, by consistent self-study, depending on the needs of different individuals.

Keywords: Hematology slides, Teaching, Examination, Learning achievement

Introduction

Hematology and the lymphoreticular system are one of compulsory subjects for preclinical-year and clinical-year medical students in Thailand. Hematology slides teaching is one part of the topics in this subject at School of Medicine, Mae Fah Luang University, Chiang Rai, Thailand, as well as most medical schools in Thailand. There are many hematologic diseases in Thailand. The benefits of peripheral blood smear (PBS) inspection are to make an accurate diagnosis of many hematologic diseases quickly, especially with basic clinical data such as history, physical examination and complete blood count (CBC). It does not require high technology, simply a light microscope and can be carried out at any time. So, inspection of PBS is very cost-effective. Many medical teachers, especially in hematology or clinical pathology, have come to realize the advantages of teaching using hematology slides, and stress to the medical students the importance of studying PBS, in order to become familiarized with abnormalities of red cells, white cells and platelets. In many medical schools, teaching this subject in both pre-clinical and clinical years, is conducted, in order to increase the experience of medical students who, following graduation, could be working in rural hospitals, with restricted resources.

The problems of teaching hematology slides relate to the restricted numbers of medical teachers compared to the number of students, time constraints and occasionally the lack of materials for teaching, such as typical slides of specific diseases. The usual method of teaching is to give each student a slide box of specific hematologic diseases and instruct the student to self-study and to ask the teacher whenever they have any questions. This method does not work effectively for many reasons. The medical students have not enough time to self-study. Some students are careless and break the slides unintentionally or loose slides for unknown reasons. There are also some students who never study the slides, a waste of time and teaching resource.

In order to make teaching hematology slides more effective and increase the concentration of students, teaching by examination is another way to increase student participation and follow the progress of learning achievement. Teaching by examination is used in many schools and universities. This style of teaching stimulates student participation. It does create some stress which normally results in increased concentration on the subject by the students. Measurement of teaching by examination may be performed using various tests, such as MCQ (multiple choice question) test, short answer or lab/OSCE (Objective Structured Clinical Examination)[1] tests. OSCE gives a limited time for the student to understand the question and the students then produce their answer in a similar short period of time. The OSCE is a feasible approach to the assessment of clinical competence for use in different cultural and geographical contexts; to assess a wide range of learning outcomes; in different specialties and disciplines; for formative and summative purposes; to assess students curriculum or an educational intervention; in the different phases of education, including the early and later years of the undergraduate curriculum; and in different health care professional settings.[2]

Method

The study was prospectively done in preclinical third year medical students, attending Mae Fah Luang University, during the Hematology and Reticular system block, in the second trimester of the 2020 academic year, taught during 11-22 January 2021. This block consisted of lectures, case discussions, case demonstrations and hematology slides. The author used examination based studies only in the hematology slides, to study this concept relating to learning achievement, after completion of the block. The study started on the fourth day of the block when the students had learned some topics during the first three days. The author uploaded all learning topics on Google classroom, in pdf format, including "How to interpret a peripheral blood smear" ahead of the actual class. In the morning of the fourth day, the author conducted the first demonstration test, which consisted of 14 PBS slides of various common hematologic diseases encountered in Thailand. The test was performed in rotating stations, 5 minutes for each station. Immediately after finishing the test, the answer was posted in Google classroom. The test score of each slide was 100, grading of the scores was 30 for the diagnosis and 70 for the explanation. The scores from the first test were evaluated and weighted 10% but not collected. After the students had marked themselves using the teacher's answers, the second test was performed in the same style, with different or the same diseases, but using different patient details, and the scores were again collected and weighted as 10% of the final total. Again, the answers were uploaded after completion of the test. So, the students had time to evaluate themselves and they could ask the teacher in detail via LINE or Google classroom. One week later, on the last day of the block, the third test was carried out with 23 PBS slides stations and the scores were weighted 10%. The teacher also uploaded the answers after the examination. The first author was the only one who examined and evaluated the answers of 32 students in all three tests.

The first test called demo consisted of 14 slides of diseases or conditions: thalassemia, viral infection (dengue fever), pancytopenia, chronic lymphocytic leukemia, essential thrombocythemia, leukemoid reaction, chronic myeloid leukemia, primary myelofibrosis, polycythemia vera, acute lymphoblastic leukemia, acute myelomonoblastic leukemia, acute promyelocytic leukemia, acute monoblastic leukemia and multiple myeloma.

The second test called quiz consisted of 14 slides of diseases or conditions: primary myelofibrosis, immune thrombocytopenia, polycythemia vera, drug-induced secondary thrombocytosis, marked eosinophilia, thalassemia, iron deficiency anemia, multiple myeloma, thrombotic thrombocytopenic purpura, G6PD deficiency with acute hemolysis, hereditary spherocytosis, autoimmune hemolytic anemia, beta thalassemia with post splenectomy.

The third test and final test consisted of 23 slides of diseases or conditions: hereditary elliptocytosis, G6PD deficiency with acute hemolysis, thalassemia, homozygous Hb E, essential thrombocythemia, chronic myeloid leukemia, immune thrombocytopenia, chronic lymphocytic leukemia, polycythemia vera, acute myeloid leukemia, lymphoplasmacytoid lymphoma, iron deficiency anemia, Hb H disease, autoimmune hemolytic anemia-cold type, lymphoma in blood smear, primary myelofibrosis, hereditary spherocytosis, acute lymphoblastic leukemia, acute promyelocytic leukemia, thalassemia trait, thrombotic thrombocytopenic purpura, viral infection (dengue hemorrhagic fever) and pancytopenia. A sample of the test is shown in Figure 1.

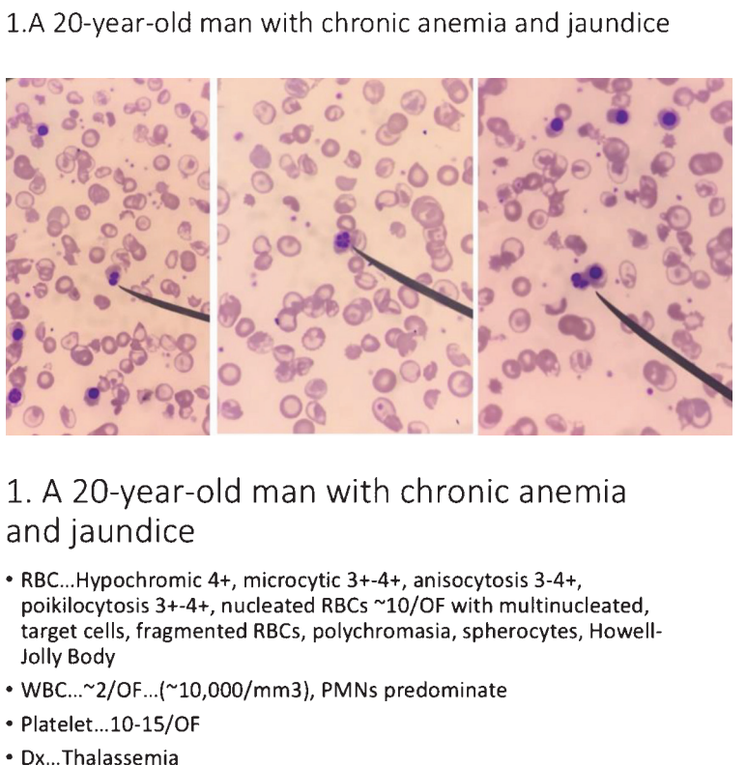


Figure 1 Sample of the test with picture and a key of the answers

Statistical analyses were conducted with the IBM SPSS Statistics 26.0 program (Armonk, New York, USA). To evaluate learning achievement of the students after each test, the scores were calculated in mean ± S.D. and plotted in graphic form. One-way ANOVA was used for comparing overall mean differences between the three tests. Post-Hoc test was used to compare mean scores differences between 1st and 2nd test, 2nd and 3rd test and finally 1st and 3rd test. Statistical significance was considered when p-value was ≤ 0.050 at 95 % confidential interval (CI).

Result

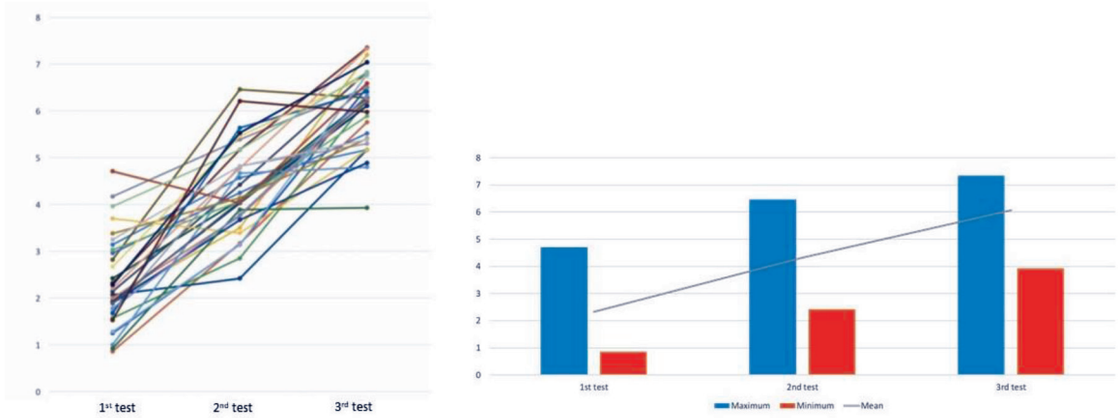
There were 32 medical students in this class, two students were retaking the year. All students completed the block and had taken the examination. The examination was weighted 60% for MCQ, hematology slides 20% (10% each for the second and the final test), case discussion 10% and attending the class 10%. For the convenience of comparison, the author calculated 10% in each test as being equal to 10 points and used this measure for statistical comparison. Thirty of 32 students (93.8%) showed improvement in their scores from the first to the second test. Thirty of 32 students (93.8%) improved their scores from the second test to the final examination. All students showed improved learning achievement from the first to the final examination, as shown in Figure 2

Mean scores and S.D.

Mean scores ± S.D. of the students from the first test (demo) was 2.33 ± 0.97 compared to 4.33 ± 0.97 in the second test (quiz) and 6.07 ± 0.81 in the final examination. The point score of each student was plotted and is shown in Figure 2. The results of mean scores and S.D. are displayed in Table 1. Minimum scores of 1st, 2nd and 3rd tests were 0.86, 2.42 and 3.93, respectively. Maximum scores of 1st, 2nd and 3rd tests were 4.71, 6.46 and 7.35, respectively.

Comparison between tests

One-way ANOVA showed that there was a significant overall difference in students' point score between the three tests ($p < 0.001$). Using Levene statistics, p-value was 0.578, indicating assumed equal variance. The Bonferroni test was used for Post-Hoc comparison, and demonstrated significant differences between the mean scores of the first and the second test (means difference 2.00, $p < 0.001$, 95 % CI 1.44 – 2.56), the second and final test (means difference 1.74, $p < 0.001$, 95 % CI 1.18 – 2.30) and the first and the final test (means difference 3.74, $p < 0.001$, 95 % CI 3.18 – 4.30). The results were shown in Table 1.



A B

Figure 2 Progression of learning achievement in 32 medical students after each test: A; all 32 students, B; maximum (blue), minimum (orange) and mean score (line) of each test

Table 1 Comparison between points of three hematology slide tests

Mean ± S.D.			One-way ANOVA				Mean points difference comparisons	p-value
1st test	2nd test	3rd test	Levene statistics	p-value of test of homogeneity of variances	F	p-value of One-way ANOVA		
2.33 ± 0.97	4.33 ± 0.97	6.07 ± 0.81	0.551	0.578	132.65	< 0.001*	2nd test > 1st test	< 0.001*
							3rd test > 2nd test	< 0.001*
							3rd test > 1st test	< 0.001*

* Significant at 95 % CI

Discussion

The study was pre-planned and prospective to evaluate the efficacy of examination-type teaching in hematology slide interpretation and initial diagnosis. In Thailand, there are some medical schools, for example Chiang Mai University in northern Thailand, using OSCE with demonstrated constructive feedback, achieving impressive results, proving that this concept is not only a good assessment tool but also is an effective teaching method in blood smear interpretative skills[3]. Another study from the Medical Education Center of Buriram Hospital in northeastern Thailand, showed that formative evaluation had enhanced the clinical skills of blood smear interpretation in medical students[4]. Initially, the author wished the students to be able to interpret abnormalities of red blood cells, white blood cells and platelets. The medical students were not yet proficient in diagnosis but there were ongoing lectures of hematology disorders later in the course. The author prepared the lecture, on how to interpret blood smear, as a self-study topic. The first test acted as a demonstration, the second test was the quiz, held during the first week of the course. The final test, the third, was held as part of the final examination. All students performed an evaluation of the teaching process after completion of the course. Most students used the key (answer) after each test to self-assess their performance, and improved their learning achievement, following each successive test. All students showed progression of their performance from the first test (mean ± S.D.) 2.33 ± 0.97 compared to 4.33 ± 0.97 in the second test and 6.07 ± 0.81 to the final test. The main key to success for this study was active learning of the students, examination-style of teaching, feedback after each test by immediately posted answers and then repeated testing. From their evaluation, the students would like to sit a smaller number of final test, because of the time involved, also they felt tired. Following student suggestion, the author plans to improve the learning process to accommodate an improved learning atmosphere in the next year by altering the timing of the test, smaller number of tests, and improved lecture on how to interpret blood smears before the test. However it is clear that retention of knowledge needs repetitive examination and/or ongoing active learning by self-study, depending on individual learning characteristics.

Conclusion

Examination-based teaching has proved to be an interesting and effective method for teaching medical students on how to interpret hematology slides. Ongoing improvement of this learning method,

following student evaluation, can make a better learning and more user friendly environment that will encourage ongoing continuous self-study and retention of knowledge.

Conflict of interest

The authors have declared no conflict of interest

Acknowledgement

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Preparation of Glass-Ceramic Biomaterial from Cow bone and Thai Mollusk shells

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Abstract:

Background: Hydroxyapatite material has excellent biocompatibility with the human body trust it has poor mechanical properties. Bioglass addition can be solving this problem. Glass-ceramic biomaterial from cow bone and Thai mollusk shells is a new material for repair the broken bone.

Objective: The purpose of this research is preparation of glass-ceramic biomaterial form cow bone and Thai mollusk shells, and to investigate their characteristics and properties.

Methods: Nano HA powder was prepared by thermal method for heating up to 950°C and ground with highspeed pot mill, beside the calcium phosphate glass was prepared by melted the mixture of CaO-P_2O_5-Na_2O at 1000°C, quenched and ground to fine powder by high speed pot mill. Then mix two type of powder in the various ratio. Samples of the composite were formed by a hydraulic press machine and sintered at temperature of 1300°C for 2 h in an electric furnace. Characterization by FTIR, XRD, SEM/EDX and UTM machine.

Results: The results from FTIR and XRD confirmed the functional groups and composition phase of HA powder that has the ion stretching vibration of hydroxyl group (OH^-), carbonate (CO_3^{2-}), and phosphate groups (PO_4^{3-}) were also observed around 1045 and 560 cm^{-1} respectively. XRD measurement showed that the ceramic contains hydroxyapatite crystals with β-tricalcium phosphate, calcium oxide and amorphous $CaPO_4$ phase. SEM measurement shows the porous, HA particles and amorphous phase. Mechanical property measurements show that the glass-ceramic contains flexural stress of 55.09 MPa.

Conclusion: In this experiment, hydroxyapatite powder and bioglass powder can be prepared for fabricating biomaterials. They could develop to apply as the production of scaffold bone, sponge bone, and bone filler.

Keywords: Hydroxyapatite, Biomaterial, Cockle shell, Bioceramic, Bioglass

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Introduction

Hydroxyapatite has been widely investigated due to their excellent biocompatibility with human's body which includes good bioactivity[1], but poor mechanical properties[2]. Many calcium phosphate-based compounds can be synthesized by chemical techniques such as co-precipitation[3,4], sol-gel synthesis[5,6], hydrothermal method[7] and thermal deposition[8]. Moreover, it can be synthesized from natural starting materials such as eggshell[9,10], coral[11] or bow bone[12,13]. These natural materials pertain some advantage properties that they inherit from the raw materials such as pore structures. Mollusk shells (aragonite) is one of the more abundant crystalline polymorphs of calcium carbonate ($CaCO_3$). It is also a good biomedical material as it is denser than calcite and could be integrated with, resolved, and replaced the bone[14].

Therefore, the aims of this work are; to fabricate dense HA ceramic from cockle shell which is the natural calcium source, and to investigate their characteristics and properties. Furthermore, the cockle shells can also be used over a wide range for medical applications

Objective

The aims of this work are to fabricate glass-ceramic from hydroxyapatite powder prepared from cow bone and co-bonding with glassy phase of calcium phosphate glass, these raw materials are the natural bone and calcium source, and to investigate their characteristics and properties.

Method

Nano HA powder was prepared by thermal method for heating up to 950°C and ground with highspeed pot mill, beside the calcium phosphate glass was prepared by melted the mixture of CaO-P_2O_5-Na_2O at 1000°C, quenched and ground to fine powder by highspeed pot mill. To fabricate the dense HA, poly vinyl alcohol (PVA, Sigma-Aldrich, Singapore) and No-Tox HD food grade grease (Bel-Ray, USA) were used as binder and lubricant. HA and $CaPO_4$ glass powder were mixed with 0.05%wt PVA and moisture content of 3% in mortar. The mixtures were uniaxial pressed at 500 psi into a disc shape (0.5 cm high × 1.5 cm diameter) by using stainless steel mold and dry pressing-hydraulic machine. The green body samples were sintered at three different temperatures of 1300°C for 2 h in electric furnace. Characterization by FTIR, XRD, SEM/EDX and UTM machine.

Results and Discussion

The FTIR spectra of the HA powder are displayed in Figure 1. The ion stretching vibration was around 3574 and 2002 cm^{-1} which confirms the presence of hydroxyl group (OH-), 1451 cm^{-1} for carbonate (CO_3^{2-}) whereas phosphate groups (PO_4^{3-}) were also observed around 1045 and 560 cm^{-1}. Figure 2 show mineral composition in cow bone powder it confirmed that is the hydroxyapatite phase.

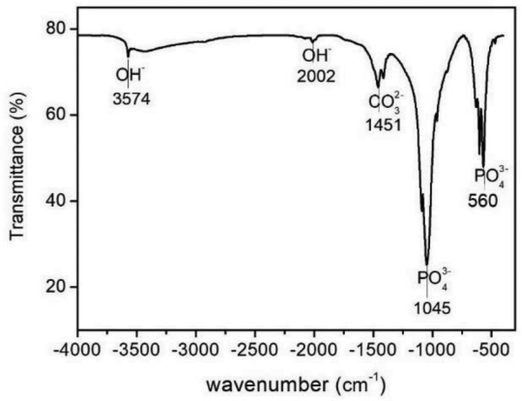

Figure 1 FTIR spectrum of synthesized HA powder.

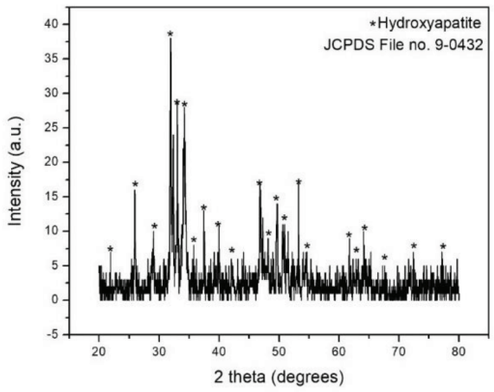

Figure 2 XRD patterns of HA powder.

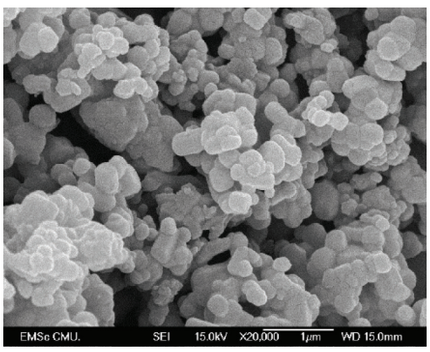

Figure 3 SEM micrograph of HA powder.

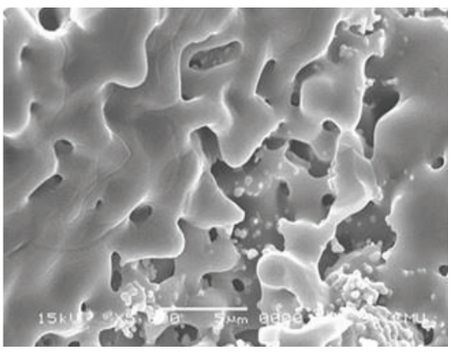

Figure 4 SEM micrograph of $CaPO_4$ glass-HA.

Figure 3 shows the morphology of HA powder that was agglomerated after the powder sample. For sintered $CaPO_4$ glass-HA ceramic shows in Figure 4 grain size range between 0.5–1 mm on the fracture surface of sample with sintered at 1300°C. An apparent porosity occupies approximately 50% of total area. The pore size ranges between 1–10 mm, while smaller pores range between 1–2 micron and bigger pore has size that range between 3–8 mm. This sintered temperature showed the formation of closed pores. Strength of materials was shown in Figure 5, the flexure stress and strain of 10% Glass: 90% HA sample, sintered at 1300°C are 55.09 MPa and 10100.19 MPa. It was lower than flexure stress and strain of dense human bone (156 MPa).

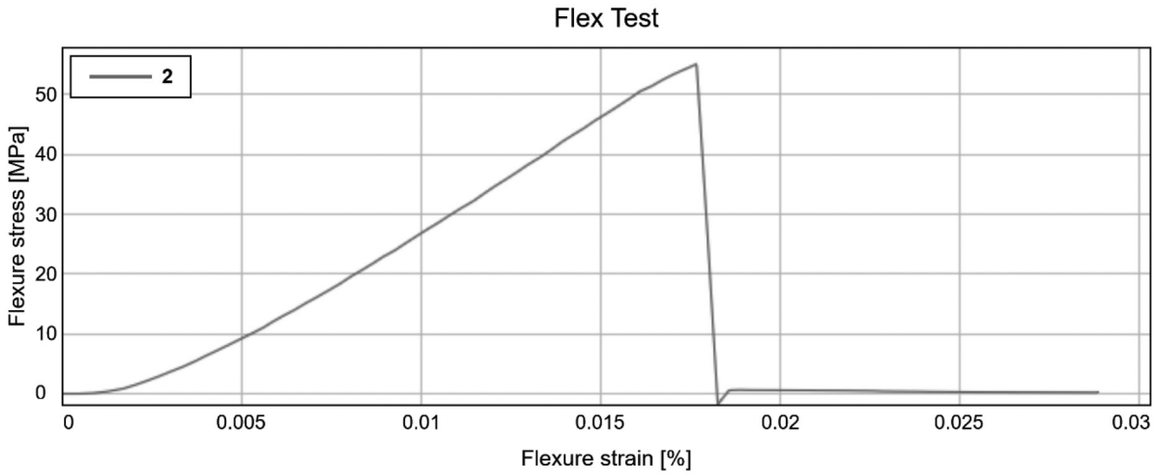

Figure 5 Flexural modulus of 10% Glass: 90% HA sample, sintered at 1300°C.

Conclusion

In this experiment, nano HA powder was prepared by thermal method and the calcium phosphate glass was prepared at 1000°C. Then, the samples of glass-ceramic composite were formed by a hydraulic press machine and sintered at temperature of 1300°C for 2 h in an electric furnace. The results from characterization technique; FTIR, XRD, SEM/EDX and UTM machine can be investigated that hydroxyapatite or HA powder and bio-glass powder were prepared from natural resource like cow bone and mollusk shells. SEM measurement shows the porous, HA particles and amorphous phase that given low mechanical property that the glass-ceramic contains flexural stress of 55.09 MPa. So, they could develop to apply as the production of scaffold bone, sponge bone, and bone filler.

Acknowledgement

This work was carried out with support from Mae Fah Luang University.

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Rehabilitation of Post-stroke Hemiplegic Patient with Integrative Medicine

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Abstract:

Numerous of stroke survivors are suffering from the body function impairments such as problems with balance or coordination, weakness, and paralysis. We describe a case in which use of integrative medicine rehabilitation for a 52-year-old female patient with ischemic stroke suffered a right hemiplegia, resulting in persistent weakness and problems with muscle control. The patient underwent sequential three healing processes including physical therapy, Thai traditional massage, and acupuncture in total of 10 times, at 2 times per week by professional practitioners. After management with this rehabilitation program, the patient had better effects on stroke recovery evaluating by The Barthel index for activities of daily living, Mini-Mental Status Examination (MMSE) Thai 2002 and muscle power grading. The outcomes of the study evidence that comprehensive integrative medicinal rehabilitation approach has multiple effects on individuals who have poststroke disability or weakness to confer greater rehabilitative benefit.

Keywords: Rehabilitation, Poststroke, Acupuncture, Thai traditional massage

Introduction

Cerebrovascular disease or stroke has become the top three leading cause of mortality in non-communicable diseases which is also the cause of disability in Thai population[1]. Ischemic stroke is the common type of stroke which can be found in elderly people as the report of the Thai Stroke Registry indicating that the mean age of patients at the onset of ischemic stroke in Thailand is around 65 years old[2]. Stroke survivors with mobility limitation, motor dysfunction, cognitive impairment, and poststroke depression are unable to live independently because of disability and leading to physical, emotional, social, and financial problems leading to the dramatic impact on their quality of life (QOL)[3]. Thus,

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the poststroke rehabilitation therapy is recommended to improve the daily activities and quality of life of these patients.

Ischemic stroke is a major type of stroke in Thai population which causes by a blockage in an artery that supplies blood to the brain, leading to damage or even death of brain cells[4]. The damage of brain cells typically impacts limbs and facial muscles of stroke survivors. It's often accompanied by muscle pain, seizures, spasticity and atrophy[5]. The clinical study has shown that the use of herbal medicine and acupuncture along with conventional rehabilitation can reduce injury level and exert a neuroprotective role in ischemic stroke patients[6]. In addition, acupuncture and Thai traditional massage have been reported to stimulate the sensory via multiple efferent pathways of neural systems[7,8]. However, early movement and exercise from physical therapy is recommended to prevent a long-term disability and promoting of neural function[9].

This case report demonstrated use of physical therapy (conventional rehabilitation) together with Thai traditional massage and acupuncture in a female patient suffered from stroke. After management with integrative approach, the patient's neurological condition, mobility and balance have improved drastically without significant side effects.

Case Presentation

A poststroke 52-year-old female (body weight, 86 kg; height, 158 cm) was diagnosed with ischemic stroke which caused her to have a right hemiplegia. Seven months before stroke, she was a physically active person, but suddenly had experienced right-sided weakness and she was admitted to the nearby hospital within 4 hours. After discharge from the hospital, she visited the local hospital for exercise therapy by physiotherapists; however, there was no significant improvement. Therefore, she decided to come to Mae Fah Luang University Hospital for rehabilitation at brain and neurology department by integrative medicine with a chief complaint of right-sided paralysis. She had a history of hypertension as well as hyperlipidemia and the physician prescribed her Enalapril 5 mg and Simvastatin 10 mg per a day as the antihypertensive and antihyperlipidemic drugs, respectively. At the first visit, she could not move her right-sided upper and lower limbs, but she could smile and raise her eyebrows symmetrically. She denied a severe headache or visual disturbances. She had a hard time for information intake and processing such as slow thinking when compared to the pre-stroke time. She could communicate by talking and writing with her left hand. The patient had elevated blood pressure at the first to third time of visiting, after that she had normal vital signs when visited for the rehabilitation program.

Investigation

Blood chemistry tests were examined at the first and tenth time of the rehabilitation program, including HbA1c, eAG, fasting plasma glucose, total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol. The Barthel index for activities of daily living, Mini-Mental Status Examination (MMSE) Thai 2002 and muscle power grading were evaluated at the first, fifth and tenth time of the rehabilitation program.

Treatment

The rehabilitation program was designed by the hospital for disability patients after stroke by integrating three medical professional fields including physical therapy, applied Thai traditional medicine and traditional Chinese medicine. The patients with disability after stroke (less than a year) who don't need any intensive care monitoring by physicians

with medically stable can apply for this integrative medicine rehabilitation program. In this study, all the techniques for the treatments were accepted as clinical care. The combination of three healing processes were delivered to the patient in the same sequence for ten times, two times per week. Initiation of the activity began with a physical therapy including exercise in the hospital gym with some equipments to improve mobility coordination, strengthen muscles and regain range of motion. This session was last for an hour under the supervision and monitoring by physiotherapists. In the next session, the patient received Thai traditional massage (Table 1). Following this, a hot herbal compress was applied to the same massage areas for approximately 40 min. The main ingredient of the herbal compress is *Zingiber cassumunar* rhizome. After that, the patient was taken through the regime of traditional Chinese medicine using acupuncture. The 0.25x40 mm fine needles were inserted into specific 11 acupuncture points (Table 2) based on YangMing meridian lines and Dumai channel of the weak or painful area for 30 min.

Table 1 Step, area and duration of Thai traditional massage treatment

Step	Area and duration
1	Apply pressure on the right leg (beginning from lower leg to upper leg then return to lower leg) and right ankle (press at anterior of ankle joint) for 6 min
2	Apply pressure on the back (along erector spinae muscle from L5 to C7) for 9 min
3	Apply pressure on the lateral side of the right leg (beginning from gluteal area to lateral side of upper and lower leg) for 5 min
4	Apply pressure on the medial side of the right leg (beginning from medial side of upper leg to lower leg) for 5 min
5	Apply pressure on the medial side of the right arm (beginning from middle of medial side of upper arm to anterior of wrist) for 5 min
6	Apply pressure on the lateral side of the right arm (beginning from middle of lateral side of upper arm to middle of lower arm) for 5 min
7	Apply pressure on the right shoulder (upper part of back at posterior shoulder) for 5 min
8	Apply pressure on the right shoulder (lateral side of lower neck and posterior of shoulder) for 5 min

Table 2 Eleven acupuncture points and areas

Point	Areas
BaiHui	Insert the needle at the top of the head at midpoint of the posterior hairline.
TongTian	Insert the needle at the midline of the anterior hairline, lateral to the midline.
Point	**Areas**
FengFu	Insert the needle at the midline of the nape of the neck, above the midpoint of the posterior hairline.
JianYu	Insert the needle at the upper border of the deltoid muscle, in the anterior border of the acromion of the right arm.
QuChi	Insert the needle at the midpoint between the lateral end of the transverse cubical crease and the lateral epicondyle of the humerus of the right arm.
WaiGuan	Insert the needle at the dorsal aspect of the forearm, above the transverse crease of the dorsum of the wrist of the right arm.
HeGu	Insert the needle at the dorsum of the hand, midway between thumb and point fingers of the right hand
HuanTiao	Insert the needle at the junction of lateral 1/3 and medial 2/3 of the line linking the prominence of greater trochanter and the sacro-coccygeal hiatus of the right buttock.
YangLingQuan	Insert the needle at the depression anterior and inferior to the small head of the fibula of the right leg.
ZuSanLi	Insert the needle at the lateral to the anterior crest of the tibia of the right leg.
JieXi	Insert the needle at the midpoint of the transverse crease of the ankle joint, between the tendons of m. extensor digitorum longus and hallucis longus of the right leg.

Results

After the patient completed five weeks of the program, most of her blood chemistry profiles were at normal range at the tenth time of the program, except HbA1c (6.5%), eAG (140.8 mg/dL), fasting plasma glucose (109 mg/dL) (Table 3). Therefore, she was recommended to the physician to monitor and manage her diabetes. Additionally, she was managed to continue with antihypertensive and antihyperlipidemic drugs including Enalapril 5 mg and Simvastatin 10 mg per a day to reduce long-term cardiovascular as well as cerebrovascular mortality and morbidity after stroke[10]. The evaluations of Barthel index and muscle power grading revealed her improvement, especially the weakened right-sided leg and arm as shown in Table 4. The Mini-Mental Status Examination (MMSE-Thai 2002) indicated the normality of her cognitive function at the end of the program.

Table 3 Blood chemistry profile of the patient

Blood tests	Results	
	1st time	**10th time**
Glucose	113 mg/dl	109 mg/dl
Cholesterol	112 mg/dL	99 mg/dL
Triglyceride	108 mg/dL	106 mg/dL
HDL-Cholesterol	52 mg/dL	58 mg/dL
LDL- Cholesterol (Direct)	51 mg/dl	56 mg/dl
HbA1c	6.7 %	6.5 %
eAG	145.9 mg/dL	140.8 mg/dL

Table 4 The evaluation of Barthel index, MMSE-Thai 2002 and motor power

Evaluation forms	Assessments					
	First		**Fifth**		**Tenth**	
Barthel index	40		80		95	
MMSE-Thai 2002	20		23		27	
Motor power of arms	Right Left	0 4	Right Left	0 4	Right Left	3 5
Motor power of legs	Right Left	0 5	Right Left	1 5	Right Left	3 5

Discussion

Most stroke survivors reach a functional plateau within the first 6 months to 1 year after stroke and are left with muscle weakness or motor impairments together with cognitive issues that can persist years in their life[11]. It has been suggested that the combination of medical therapies potential for further recovery remains in stroke survivors who have reached such a plateau and that may accelerate this recovery in greater context[6]. Physical therapy (conventional rehabilitation) together with Thai traditional massage and acupuncture provide the potential therapies to facilitate the recovery for the patient. In this study, the results from the Barthel index for activities of daily living, Mini-Mental Status Examination Thai 2002 and muscle power grading show that integrative medicine is possible for the patient with right hemiplegia to effectively improve the fine motor control and cognitive function through physical therapy together with Thai traditional massage and acupuncture. Another study shows that the application of physical therapy, Thai traditional massage, and herbal medicine assisted the improvement of daily function, pain, mood and sleep pattern in stroke patients[12]. Additionally, acupuncture which has been approved by WHO has shown the potential effect on ischemic stroke

through modulation of different mechanisms originating in the central nervous system[8]. In term of safety for the use of acupuncture and Thai traditional massage, a rare incidence of adverse events or even mild symptoms were reported[13]. The external use of the hot herbal compress is considered safety; however, allergic reaction might occur with some patients. Moreover, as long as acupuncture is performed by trained practitioners using the clean needle technique, it is usually a safe procedure[14]. Taken together, the beneficial results on all outcome measurements during the follow-up period indicated that this integrative rehabilitation approach may contribute to many aspects of stroke recovery.

Conclusion

The results in this case report are promising in poststroke rehabilitation as well as for individuals with preexisting disabilities or neurological conditions that may benefit for them. Moreover, future clinical trials are required to confirm this assertion.

Conflict of interest

The author has declared no conflict of interest.

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Case Report GMSMJ Greater Mekong Subregion Medical Journal

A Young Man with High-grade Fever and HIV infection

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Abstract:

A 23-year-old man, a hilltribe in northern Thailand with HIV infection but poor compliance to antiviral treatment presented with acute onset of fever, fatigue with palpitation. He also experienced easily fatigue and tiredness for 1 month. He went to local hospital and was treated symptomatically without any improvement. Then he went to another hospital for management. Initial investigation showed anemia and thrombocytopenia. The bone marrow examination revealed yeast-like microorganism with binary fission. Final diagnosis of disseminated talaromycosis (penicillosis) was made. So, in case of fever of unknown origin in HIV-infected patients, bone marrow examination should be performed which can be helpful for definite diagnosis.

Keywords: HIV infection, Disseminated talaromycosis, High-grade fever

Introduction

Untreated HIV-infected patients usually have fever which some of them are initially unable to identify the causes. Most of them are from opportunistic infection (OI) due to bacteria, viruses, fungi, and protozoa. In Thailand, there are high incidence of some OI such as mycobacterial infection (tuberculosis and atypical mycobacterium), cryptococcal meningitis, disseminated histoplasmosis and talaromycosis (penicillosis). Although other infections such as Pneumocystis carinii, toxoplasmosis, parvovirus B19 and CMV infection can be encountered. Besides infection, HIV-infected patients are also having higher incidence of lymphoma which can cause fever. The problem of physicians taking care of these patients is unable to diagnose the causative infection. Some physicians use therapeutic diagnosis with antituberculosis drugs. This method can cause delayed diagnosis and increased morbidity and mortality. Prompt investigation with rapid result is needed especially in resource-restricted rural hospital.

Case Presentation

A 23-year-old man, a hilltribe in northern Thailand with HIV infection but poor compliance to antiviral treatment presented with acute onset of fever, fatigue with palpitation and non-productive cough for 1 month. He had no diarrhea, nausea

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or vomiting. He went to the local hospital and received symptomatic treatment without improvement. So, he came to another hospital for management. He had previous admission to the hospital 3 months prior to this admission because of anorexia, weight loss, non-productive cough, low grade fever and was diagnosed AIDS with pneumocystis carinii infection. On admission to this hospital, vital signs showed body temperature of 37.8°C (after taking paracetamol), heart rate 120/minute, blood pressure 113/74 mmHg, respiration 20/min, height 159 cm, weight 44 kgs with BMI of 17.4 kg/m^2. He was moderately anemic without jaundice. He had no palpable lymphadenopathy but palpable liver and spleen 4 cm. and 6 cm. respectively below costal margins. No any skin lesion was detected. Investigation revealed Hct 26%, Hb 8.0 g/dL, WBC 5.39 x 10^9/L, PMN 76%, L 9%, M 3%, E 11%, platelet 100.0 x 10^9/L, MCV 70 fL, MCH 23 pg, BUN 14 mg/dL, Cr 0.77 mg/dL, GFR 128 mL/min, Na 126 mmol/L, K 3.9 mmol/L, Cl 94 mmol/L, CO2 25 mmol/L, total protein 6.6 g/dL, albumin 3.2 g/dL, globulin 3.4 g/dL, total bilirubin 0.3 mg/dL, direct bilirubin 0.1 mg/dL, AST 36 U/L, ALT 23 U/L, ALP 59 U/L, CD4 36/μL. Chest x-ray: no pulmonary infiltration. Ultrasound of whole abdomen: hepatomegaly with diffuse increased parenchymal echogenicity and splenomegaly with wedge shaped patchy hypoechogenicity, possible infarction and several enlarged lymph nodes along periportal regions. Review of blood smear found mild hypochromic red blood cells with frequent polychromasia. Bone marrow aspiration and biopsy was performed. The finding revealed hypercellular bone marrow with intracellular yeast-like organisms, some had binary fission compatible with talaromycosis (penicillosis) as shown in Figure 1. So, the patient was started with Amphotericin B 1 mg/kg on the admission day. On the following day, he was doing better with fever down. He subsequently received oral fluconazole. A week later, blood culture for fungus was reporting *Penicillium marneffei*. The bone marrow biopsy came back 1 week later and revealed talaromyces as in Figure 2, 3 and 4.

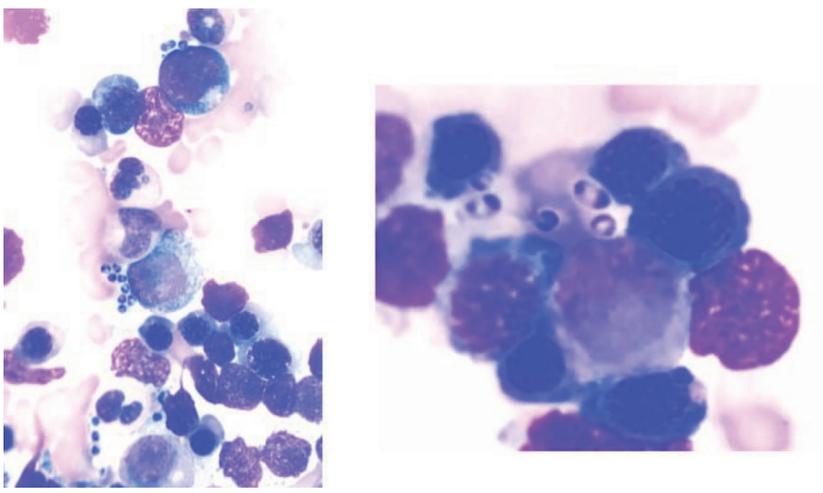

Figure 1 Bone marrow smear shows intracellular yeast-like organisms with binary fission consistent with talaromycosis (penicillosis). (left x 40, right x100)

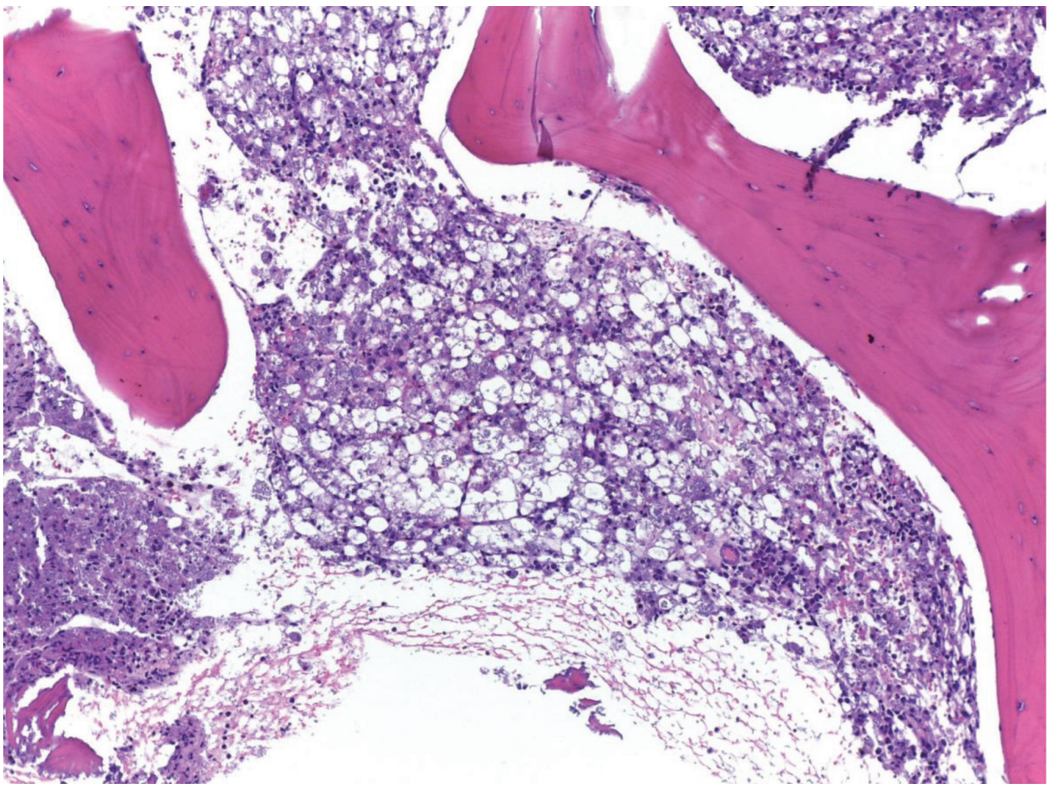

Figure 2 The bone marrow biopsy x40 shows marked hypercellularity.

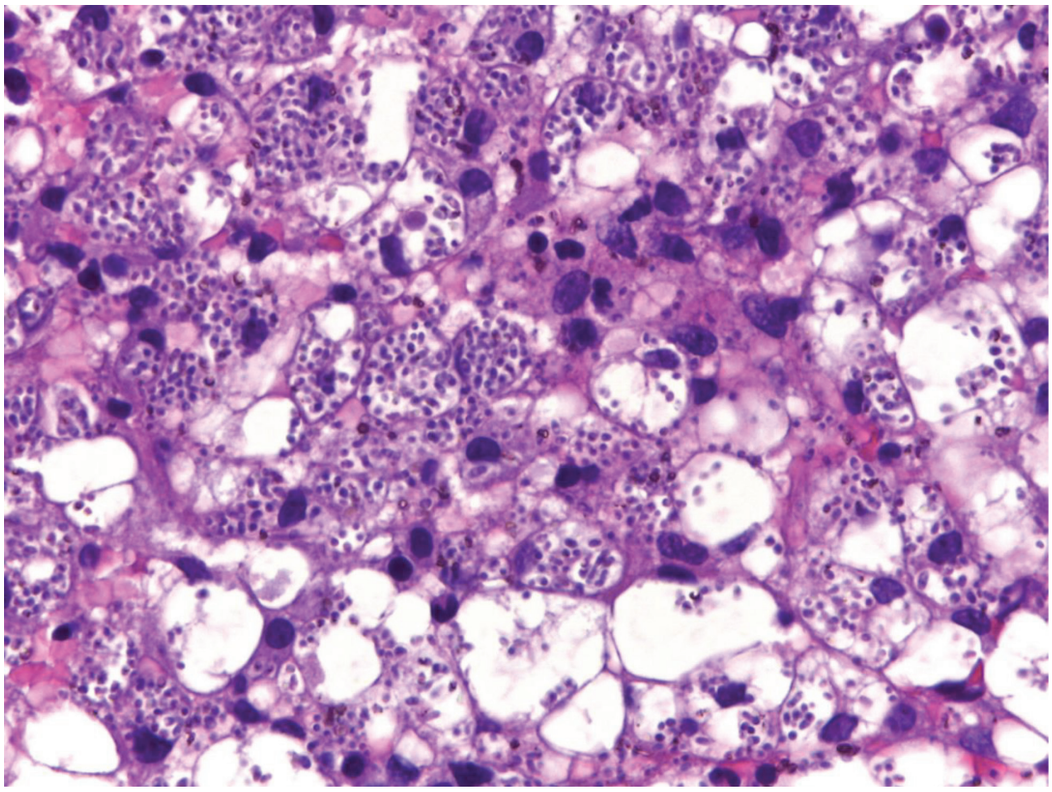

Figure 3 The bone marrow biopsy x 100 shows many aggregates containing many yeast-like particles.

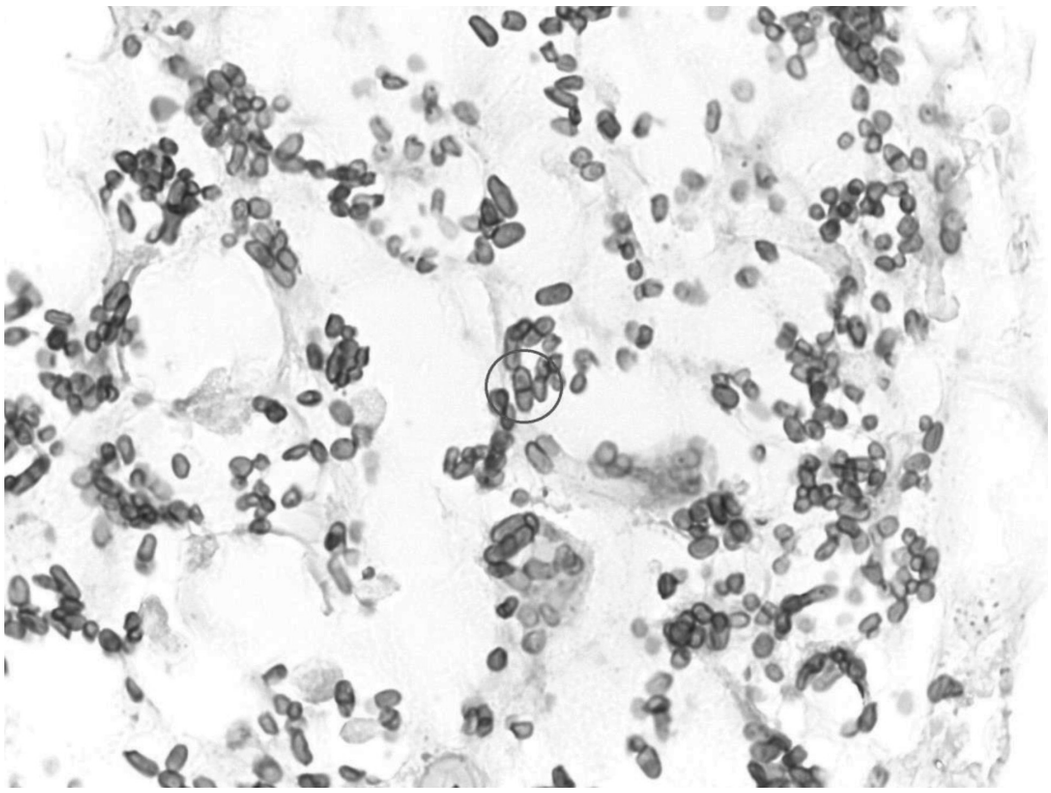

Figure 4 GMS stain in the bone marrow highlights many oval-shaped yeasts without budding but occasional binary fission (in circle).

Discussion

This patient was a 23-year-old hilltribe man with acute onset of fever, fatigue with palpitation. He was also diagnosed HIV infection with possible pneumocystis carinii pneumonia 3 months ago with poor compliance to antiretroviral treatment. By physical examination, he had anemia, hepatosplenomegaly without any obvious skin lesions. So, differential diagnosis should be opportunistic infections such as mycobacterial infection both TB (tuberculosis) and MAC (mycobacterium avium complex), cryptococcal infection although having no significant headache of stiff neck, other disseminated fungal infection and because he had intraabdominal lymphadenopathy, lymphoma should be in the differential diagnosis. Initial CBC showed anemia and thrombocytopenia, although blood smear did not show myelophthisis, this could not totally exclude infection or malignancy in the bone marrow. If we were waiting for culture result or therapeutic trial with antituberculosis drugs, this could be more harmful because of delayed diagnosis and adverse drug reaction. Bone marrow examination in this patient was justified and could make rapid diagnosis.

Talaromycosis[1] (formerly Penicilliosis[2]) is an infection caused by the fungus *Talaromyces marneffei*. Talaromycosis commonly affects people who live in Southeast Asia[3], southern China[4], or eastern India. Most people who get talaromycosis have defective immune function, especially HIV/AIDS[5]. In Thailand, this infection usually found in northern part[6] such as Chiang Rai or Chiang Mai. Patients with this infection mostly experience high-grade fever, anorexia, weight loss, painless skin lesion. Some patients have cough,

difficulty breathing, diarrhea, abdominal pain, lymphadenopathy and palpable hepatosplenomegaly[7].

In people with HIV-infected patients, talaromycosis is more likely to spread through the blood and affect the whole body. Skin lesions due to talaromycosis usually have a small dent in the center. In non-HIV infected patients, talaromycosis commonly affects the lungs, liver, and mouth. Sometimes it spreads through the blood and affects the whole body. Patients with other immune dysfunction such as cancer, organ transplant, adult-onset immunodeficiency syndrome and other autoimmune diseases can be infected with talaromycosis[8].

Bone marrow examination can identify this organism in macrophages or neutrophils. Histoplasma and talaromyces are yeast like organism. Both look similar, but talaromyces has typical binary fission. However, definite diagnosis requires fungal culture.

Talaromycosis is usually treated with amphotericin B for two weeks, followed by itraconazole or voriconazole given by mouth for 10 weeks. Patients with HIV infection should receive antiretroviral one week after starting amphotericin B[9].

Conclusion

The authors reported case of disseminated *Talaromyces (Penicillium) marneffei* infection in HIV-infected patient. We suggest bone marrow examination and culture for diagnosis fever of unknown origin in this setting.

Conflict of interest

The author has declared no conflict of interest.

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Antibacterial Activity against *Streptococcus mutans* of Brass-Ash-Derived Zinc Oxide Nanoparticles

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Abstract:

Background: In recent years, popularity has been grown into management of industrial wastes or chemical by-products, and brass ash is the one to be in consideration. With its high amount of zinc oxide (ZnO), this fly ash is able to be processed for ZnO which provides a lot of useful medical applications, especially antimicrobial activity against certain kinds of bacteria.

Objective: This study aims to examine whether antibacterial activity against *Streptococcus mutans* (S. mutans) of waste-derived ZnO is obtained by which size of the milled particles.

Method: Once the ash was obtained, it was then purified for ZnO using chemical precipitation method. Subsequently, purified ZnO (S_0) was milled in a high energy ball miller under a variety of milling durations: untreated, 0.5-hour milled ($S_{0.5}$), and 1.0-hour milled (S_1). Characterization of ZnO was done through x-ray fluorescence (XRF) analyzer for element composition, x-ray diffraction (XRD) machine for determination of crystallographic parameters, as well as scanning electron microscope (SEM) for particle size distribution. Purified and milled ZnO was, at last, tested for its antibacterial activity against *S. mutans* using microbroth dilution method compared to commercial ZnO (C), and the result was interpreted using a microplate reader.

Results: ZnO at 99% purification was synthesized in the process. Its size means were 123.99 nm for untreated ZnO, and 104.41 nm and 76.22 nm respectively after being milled. Of all the sizes compared, optimal antibacterial activity, eventually, was acquired from ZnO in size of 76.22 nm (milled for an hour), which its minimal inhibitory concentration (MIC) was 28.125 μg/ml.

Conclusion: The smaller the size of ZnO, the greater the antibacterial activity is. Aside from the main objective, chemical precipitation process could purify ZnO and size distribution was affected by milling time. For further study, the author suggests that synthesized ZnO

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should be incorporated into ZnO-containing dental products and be tested for its significant change in properties. Also, testing of brass-ash-derived ZnO on other bacteria is essential for further research.

Keywords: Zinc oxide, *Streptococcus mutans*, Antibacterial activity

Introduction

Brass, nowadays, is a widely-used alloy owing to its advantages, for example, excellent corrosive resistance, polishing and finishing characteristic, and easy manufacturing process[1]. Nevertheless, during smelting, a large portion of brass slags are formed, causing a leak into environment if not properly managed. When milled and pulverized, these slags are typically called "brass ash" or "brass fume", containing a large amount of ZnO, a smaller amount of CuO, and some other impurities—e.g., SiO_2 or PbO[2]. Despite their harmfulness, recovery of valuable compounds present is feasible through several methods[3-6].

As brass ash has a great deal of ZnO contents, when purified, applications of ZnO come into varieties. To illustrate, it is used in ceramic products, electronic compartments, additive in fertilizers or animal feed, vitamin supplements, and sunscreen[7]. In biomedical aspects, it is also applied to aid bio-imaging, bio-sensors, and delivery of drugs or genes[8]. Similarly, with or without the presence of light activation, ZnO, specifically in nanoscale, is synthesized to utilize its antimicrobial effect and it is proved to be effective by lots of researches[9-11]. Many of dental products are a good example of an application of this property: for instance, intermediate restorative material (IRM) applies the property in combination with the strength of ZnO as a temporary tooth restoration with bactericidal activity[12]. Endodontists are also another example to apply the powder as well as eugenol as a sealer during tooth obturation with the effect[13].

Major antibacterial mechanisms of ZnO are reported in the following 3 methods, including release of reactive oxygen species (ROS), Zn^{2+} release, and direct contact to bacterial cell. These mechanisms can cause damage to cell structures critically, leading to leakage of cellular contents and eventually cell death[9].

Even if ZnO is claimed to provide antibacterial effect against various species of bacteria, it is not yet elucidated that the one synthesized from brass ash and milled for different sizes are effective against *S. mutans*, the strain which is significantly attributed to dental caries among young population[14].

Caries mechanisms of *S. mutans* are initiated when acquired pellicle starts coating the tooth surface. This, then, allows some kind of bacteria, including *S. mutans*, to colonize and grow. The strain, in the presence of fermentable carbohydrates, is capable of producing acid, thereby creating acidic environment—which cariogenic bacteria favor, and demineralizing tooth structure—which, in the end, leads to dental caries[15].

As a result, this research is aimed initially to derive ZnO nanoparticles (NP) from industrial brass ash using chemical precipitation technique, subsequently to mill the powder under varied conditions so that the particles are at different sizes, and to test antibacterial activity of ZnO against *S. mutans*. using microdilution in the end[16,17].

Method and Experimental Procedure

1) Purification and Synthesis of ZnO

1.1) Analyze components contained

in a sample of the obtained brass ash with XRF spectrophotometer (HORIBA, MESA 500-W) and its corresponding software (MESA).

1.2) 40 g of brass ash powder is then leached with 250 ml of 23% w/w NH_4Cl solution, producing the solution with undissolved precipitate. This step is done under constant stirring for 1 hour and a temperature of approximate 90ºC using a magnetic hot plate stirrer (Wisdom LABORATORY INSTRUMENTS, WiseStir).

1.3) A vacuum filter (IM-TECH, 2 Stages Vacuum Pump) is applied with suction flask and Buchner funnel to filter out the precipitate from step 1.2). Note that the filter papers (Whatman, FILTER PAPER 4) should be moistened with a few drops of water to ensure vacuum filtration.

1.4) Add 3 g of finely powered Zn metal to the remaining liquid part.

1.5) Add 5-10 drops of 1000 ppm polymethyl methacrylate (PMMA) as an anti-flocculating agent.

1.6) Again, a vacuum filter is applied to filter out the precipitate (occurred in 1.4).

1.7) The Zn-containing solution is left to cool at room temperature (25ºC to 35ºC) for another 2 hours.

1.8) Filter the suspension to collect the white precipitate formed.

1.9) Re-suspend the solid in water at around 90ºC with continuous stirring for a period of 1 hour.

1.10) After alcohol wash and repeated filtration, the solid is then dried at 70ºC in a hot air oven (memmert, UF 110) for 1 hour. The white precipitate in this step is expected to be ZnO.

1.11) Re-analyze the precipitate using x-ray fluorescence spectrophotometer for its elemental composition and x-ray diffractometer (PANalytical, X' Pert-PRO MPD) in combination with X'Pert High-Score Plus programme for its crystallographic parameters. Be reminded that commercial ZnO (M Dent, Zinc Oxide) is also included for examination as a reference.

2) Size Particulation for ZnO NP[18-20]

2.1) Measure initial particle size of purified ZnO powder using SEM (LEO, LEO 1450VP) with an aid of ImageJ programme.

2.2) Equally divide the mass of ZnO into 3 groups: one for no treatment (S_0) with commercial ZnO (C) as a reference, and the other two for 0.5-hour ($S_{0.5}$) and 1.0-hour milling time (S_1) orderly. Of the 2 groups, each will be milled using a high-energy ball mill under the following specifications: 125-ml ZrO_2 container, 0.3-mm-diameter ZrO_2 milling ball (275 g in total), spin at 1200 rpm, and 95% v/v Ethanol (C_2H_5OH) as medium.

2.3) To isolate milled particles from milling balls, a sieve of a size smaller than the milling balls is used. Here, a 250-micron mesh aperture is applied to complete this step.

2.4) It is suggested that the milled particles be kept in a hot air oven until they are dry or free of the milling media.

2.5) Similarly to 2.1), re-analyze the size distribution of milled ZnO particles from SEM images using ImageJ program.

3) Testing of ZnO NP for Antibacterial Activity against *S. mutans* Using Microbroth Dilution Method[16,17]

Antibacterial activity testing in this research is modified from the suggested guidelines and protocols from Clinical and Laboratory Standards Institute (CLSI) and uses MIC as a representation of antibacterial activity using microdilution[16,17]. For preparation of antibacterial agents in detail see Table 1

Table 1 Selected scheme of preparation of antibacterial agents

Groups/ Conditions	Agents/Code	Solvents	Diluents	Concentration (µg/ml)
Group A: Primary Test and Report	**Ampicillin** T.P.DRUG LABORATORIES (1969), STERILE AMPICILLIN SODIUM, 100% equivalent to ampicillin	0.9% NaCl	0.9% NaCl	8
Group B: Primary Test and Report Selectively	**Vancomycin** SIGMA Life Science, Vancomycin hydrochloride from *Streptomyces orientalis*, 90% equivalent to vancomycin	Water	Water	16
Commercially Made	C	DMSO	DMSO	7200
Brass-Ash-Derived	S_0	DMSO	DMSO	7200
	$S_{0.5}$	DMSO	DMSO	7200
	S_1	DMSO	DMSO	7200

Abbreviations: NSS, normal saline solution; DMSO, dimethyl sulfoxide

Results

1) XRF and XRD results

Table 2 XRF results

Type of ZnO	Result			
	Element	**Mass (%)**	**Oxide Form**	**Mass (%)**
Brass Ash	Zn	73.024	ZnO	90.897
	O	18.910	-	-
	Cu	3.335	CuO	4.175
	Cl	3.151	-	3.151
	Pb	1.191	PbO	1.283
	Ca	0.148	CaO	0.207
	Br	0.133	-	0.133
	Fe	0.107	Fe2O3	0.153
C	Zn	79.993	ZnO	99.573
	O	19.690	-	-
	Na	0.276	Na2O	0.373
	Fe	0.026	Fe2O3	0.037
	Ni	0.014	NiO	0.018
S_0	Zn	79.722	ZnO	99.235
	O	19.513	-	-
	Cl	0.765	-	0.765

From Table 2, XRF analysis of the ash manifests lesser percentage of ZnO (90.897%) when compared with that of C and S_0. XRF result also indicates that the reference powder contains 99.573% of ZnO by mass with some other compounds accounting for 0.427%. Likewise, that of S0 shows 99.235% purity with only 0.765% chlorine contamination.

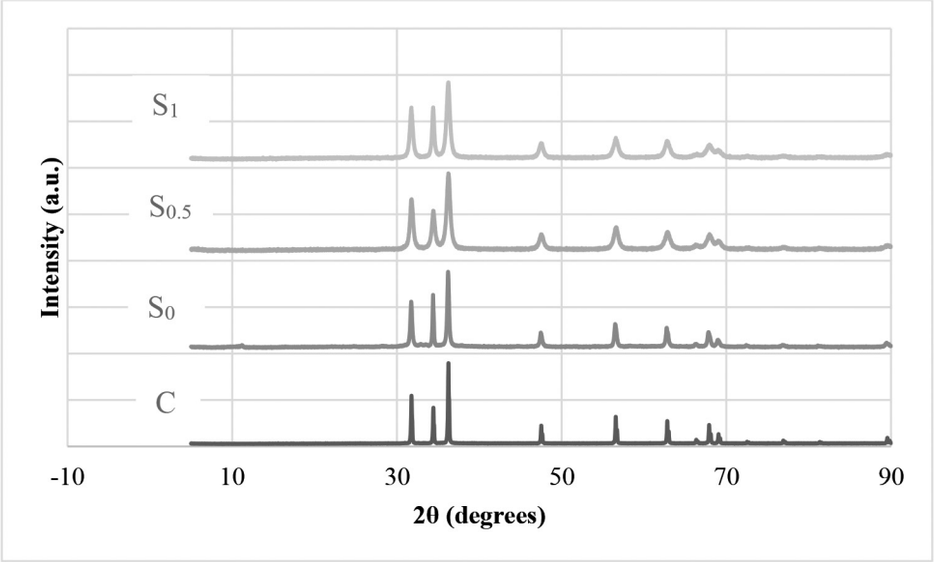

Figure 1 Stacked XRD results

The investigation is carried out with usage of Cu Kα radiation (1.5418740 Å) using continuous Gonio scanning mode at 2θ ranging from 4.9980 to 89.9999. After examination of 2 kinds of ZnO—one for reference (shown as blue, C) and the other 3 for synthesized ZnO (S_0, $S_{0.5}$, S_1), their XRD patterns match well with the reference peaks. These sorted diffractograms reveal that both types of ZnO are indexed greatly to hexagonal wurtzite structure with the same crystallographic parameters.

2) ZnO Size Distribution and Related MIC Interpretations

Table 3 ZnO size distribution with corresponding MIC

ZnO Category	Mean Size (nm)	Interpreted MIC (μg/ml)
C	194.450	112.5
S_0	123.991	112.5
$S_{0.5}$	104.410	56.25
S_1	76.221	28.125

Based on the information provided by Table 3, trend of MIC can be interpreted that when particle size of ZnO is reduced, its MIC, which represents the antibacterial activity, tends to increase. As the size is reduced to 76.221 nm, the MIC is optimal (28.125 μg/ml, n = 3)

Discussion and Conclusion

Chemical precipitation technique used to purify ZnO yields high content of ZnO precipitate up to 99% purification with its dimensions nearly reaching nanoscale[21]. To optimize the purification procedure, other remaining precipitates, acquired through vacuum filtration, should be purified

additionally so that it makes purification by-products of use and applicable[3,4]. For analytical process, it is suggested that XRF and XRD examinations of these precipitates as well as the latter investigation for brass ash should be incorporated for detailed verifying methods. Note that every device and instrument used should be calibrated with its own reliable appliances before used, and the notion is also included for other steps performed in the experiment. For large scale production of ZnO from brass ash, even if this experiment exhibits cost-effectiveness in the process, productivity and time taken for synthesis, still, are supposed to be improved.

Mean size of milled ZnO is affected by total milling time the powder is milled in the machine[20]. Nonetheless, it cannot be concluded that the size is, as well, influenced by milling ball size, ball material, etc. Thus, the author recommends studying other effects on size of the compound for the purpose of generating guided milling conditions to produce desired particle size. As there are many shapes of obtained ZnO, when the size, especially in rod shape, is reported, the author is suggested that length of the particle should be measured and added to the result in addition to its width. For more convenient and acceptable way of size interpretation, the author advises that a particle size analyzer be applied[22]. This will improve the accuracy level of size distribution as crystallite size, not agglomerated particle size, is appraised. Finally, a change in color of ZnO from white to soft yellow is noticed together with an increase in surrounding temperature after milled. It is discussed that whether hydrogen incorporation, oxygen deprivation, or a combination of multiple factors is the cause of this thermo-chromic phenomenon of ZnO[23].

According to the evaluation of antibacterial activity against *S. mutans*, the compound at all sizes exhibits the activity. This is especially the greatest for ZnO powder with the smallest size compared (S_1), revealing that its optimal MIC is equal to 28.125 μg/ml. The MIC results of these powders correlate with those present in other literatures, which the value ranges from 0.156 to 806.18 μg/m[9,10]. However, the process of antibacterial testing used in those studies is completely not the same as the one used in this study, and minimal bactericidal concentration (MBC) is suggested to be incorporated. More replications as well as controls should be performed to acquire a more dependable result. Additionally, it is still dubious about how MIC is interpreted if there is no clear inoculated well present, as ZnO and the inoculum both can make the medium turbid. Another recommendation for this part of experiment is to apply CAMHB with supplemental 2 – 5% v/v LHB following the suggested protocol instead of MHB used here; yet, there some reasons why MHB is used as medium of choice for susceptibility testing. To begin with, it shows acceptable batch-to-batch reproducibility for susceptibility testing. In addition, not only does it support satisfactory growth of most pathogens, it is also low in inhibitors that affect the results of some drugs. In the last place, a large body of data and experience has been gathered about tests performed with this medium[16].

Further study in this field of research is suggested on in-vivo antibacterial testing or testing of a change in properties after ZnO NP synthesized from brass ash is integrated into dental products or some other medical applications as additional features of nanoparticles have been widely reported due to their larger surface area to volume ratio[5,8]. Besides, even if antibacterial activity of ZnO against various strains of bacteria is reported, the test of these brass-ash derivatives against other bacteria, except *S. mutans*, is somehow recommended[9-11].

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in a sample of the obtained brass ash with XRF spectrophotometer (HORIBA, MESA 500-W) and its corresponding software (MESA).

1.2) 40 g of brass ash powder is then leached with 250 ml of 23% w/w NH_4Cl solution, producing the solution with undissolved precipitate. This step is done under constant stirring for 1 hour and a temperature of approximate 90ºC using a magnetic hot plate stirrer (Wisdom LABORATORY INSTRUMENTS, WiseStir).

1.3) A vacuum filter (IM-TECH, 2 Stages Vacuum Pump) is applied with suction flask and Buchner funnel to filter out the precipitate from step 1.2). Note that the filter papers (Whatman, FILTER PAPER 4) should be moistened with a few drops of water to ensure vacuum filtration.

1.4) Add 3 g of finely powered Zn metal to the remaining liquid part.

1.5) Add 5-10 drops of 1000 ppm polymethyl methacrylate (PMMA) as an anti-flocculating agent.

1.6) Again, a vacuum filter is applied to filter out the precipitate (occurred in 1.4).

1.7) The Zn-containing solution is left to cool at room temperature (25ºC to 35ºC) for another 2 hours.

1.8) Filter the suspension to collect the white precipitate formed.

1.9) Re-suspend the solid in water at around 90ºC with continuous stirring for a period of 1 hour.

1.10) After alcohol wash and repeated filtration, the solid is then dried at 70ºC in a hot air oven (memmert, UF 110) for 1 hour. The white precipitate in this step is expected to be ZnO.

1.11) Re-analyze the precipitate using x-ray fluorescence spectrophotometer for its elemental composition and x-ray diffractometer (PANalytical, X' Pert-PRO MPD) in combination with X'Pert High-Score Plus programme for its crystallographic parameters. Be reminded that commercial ZnO (M Dent, Zinc Oxide) is also included for examination as a reference.

2) Size Particulation for ZnO NP[18-20]

2.1) Measure initial particle size of purified ZnO powder using SEM (LEO, LEO 1450VP) with an aid of ImageJ programme.

2.2) Equally divide the mass of ZnO into 3 groups: one for no treatment (S_0) with commercial ZnO (C) as a reference, and the other two for 0.5-hour ($S_{0.5}$) and 1.0-hour milling time (S_1) orderly. Of the 2 groups, each will be milled using a high-energy ball mill under the following specifications: 125-ml ZrO_2 container, 0.3-mm-diameter ZrO_2 milling ball (275 g in total), spin at 1200 rpm, and 95% v/v Ethanol (C_2H_5OH) as medium.

2.3) To isolate milled particles from milling balls, a sieve of a size smaller than the milling balls is used. Here, a 250-micron mesh aperture is applied to complete this step.

2.4) It is suggested that the milled particles be kept in a hot air oven until they are dry or free of the milling media.

2.5) Similarly to 2.1), re-analyze the size distribution of milled ZnO particles from SEM images using ImageJ program.

3) Testing of ZnO NP for Antibacterial Activity against *S. mutans* Using Microbroth Dilution Method[16,17]

Antibacterial activity testing in this research is modified from the suggested guidelines and protocols from Clinical and Laboratory Standards Institute (CLSI) and uses MIC as a representation of antibacterial activity using microdilution[16,17]. For preparation of antibacterial agents in detail see Table 1

Table 1 Selected scheme of preparation of antibacterial agents

Groups/ Conditions	Agents/Code	Solvents	Diluents	Concentration (µg/ml)
Group A: Primary Test and Report	**Ampicillin** T.P.DRUG LABORATORIES (1969), STERILE AMPICILLIN SODIUM, 100% equivalent to ampicillin	0.9% NaCl	0.9% NaCl	8
Group B: Primary Test and Report Selectively	**Vancomycin** SIGMA Life Science, Vancomycin hydrochloride from *Streptomyces orientalis*, 90% equivalent to vancomycin	Water	Water	16
Commercially Made	C	DMSO	DMSO	7200
Brass-Ash-Derived	S_0	DMSO	DMSO	7200
	$S_{0.5}$	DMSO	DMSO	7200
	S_1	DMSO	DMSO	7200

Abbreviations: NSS, normal saline solution; DMSO, dimethyl sulfoxide

Results

1) XRF and XRD results

Table 2 XRF results

Type of ZnO	Result			
	Element	**Mass (%)**	**Oxide Form**	**Mass (%)**
Brass Ash	Zn	73.024	ZnO	90.897
	O	18.910	-	-
	Cu	3.335	CuO	4.175
	Cl	3.151	-	3.151
	Pb	1.191	PbO	1.283
	Ca	0.148	CaO	0.207
	Br	0.133	-	0.133
	Fe	0.107	Fe2O3	0.153
C	Zn	79.993	ZnO	99.573
	O	19.690	-	-
	Na	0.276	Na2O	0.373
	Fe	0.026	Fe2O3	0.037
	Ni	0.014	NiO	0.018
S_0	Zn	79.722	ZnO	99.235
	O	19.513	-	-
	Cl	0.765	-	0.765

From Table 2, XRF analysis of the ash manifests lesser percentage of ZnO (90.897%) when compared with that of C and S_0. XRF result also indicates that the reference powder contains 99.573% of ZnO by mass with some other compounds accounting for 0.427%. Likewise, that of S0 shows 99.235% purity with only 0.765% chlorine contamination.

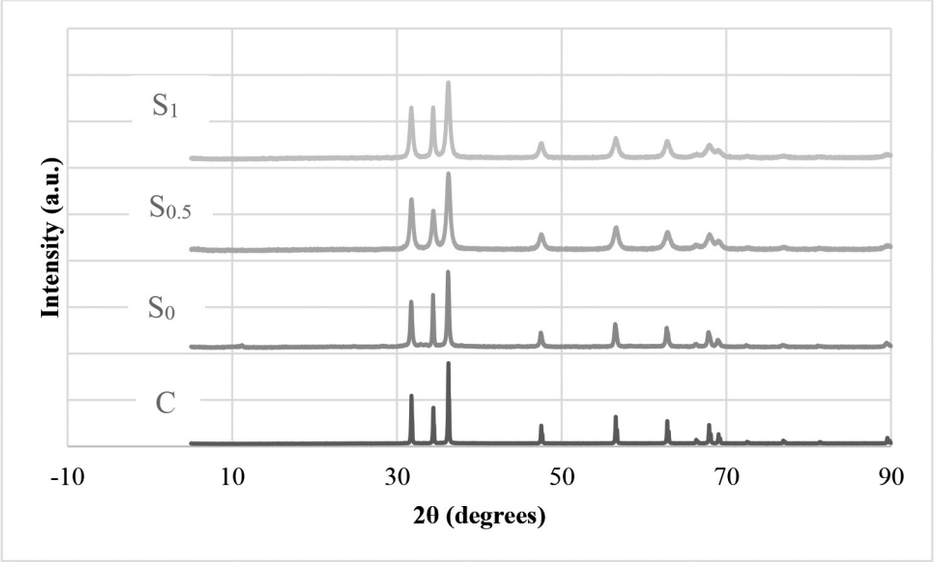

Figure 1 Stacked XRD results

The investigation is carried out with usage of Cu Kα radiation (1.5418740 Å) using continuous Gonio scanning mode at 2θ ranging from 4.9980 to 89.9999. After examination of 2 kinds of ZnO—one for reference (shown as blue, C) and the other 3 for synthesized ZnO (S_0, $S_{0.5}$, S_1), their XRD patterns match well with the reference peaks. These sorted diffractograms reveal that both types of ZnO are indexed greatly to hexagonal wurtzite structure with the same crystallographic parameters.

2) ZnO Size Distribution and Related MIC Interpretations

Table 3 ZnO size distribution with corresponding MIC

ZnO Category	Mean Size (nm)	Interpreted MIC (μg/ml)
C	194.450	112.5
S_0	123.991	112.5
$S_{0.5}$	104.410	56.25
S_1	76.221	28.125

Based on the information provided by Table 3, trend of MIC can be interpreted that when particle size of ZnO is reduced, its MIC, which represents the antibacterial activity, tends to increase. As the size is reduced to 76.221 nm, the MIC is optimal (28.125 μg/ml, n = 3)

Discussion and Conclusion

Chemical precipitation technique used to purify ZnO yields high content of ZnO precipitate up to 99% purification with its dimensions nearly reaching nanoscale[21]. To optimize the purification procedure, other remaining precipitates, acquired through vacuum filtration, should be purified

additionally so that it makes purification by-products of use and applicable[3,4]. For analytical process, it is suggested that XRF and XRD examinations of these precipitates as well as the latter investigation for brass ash should be incorporated for detailed verifying methods. Note that every device and instrument used should be calibrated with its own reliable appliances before used, and the notion is also included for other steps performed in the experiment. For large scale production of ZnO from brass ash, even if this experiment exhibits cost-effectiveness in the process, productivity and time taken for synthesis, still, are supposed to be improved.

Mean size of milled ZnO is affected by total milling time the powder is milled in the machine[20]. Nonetheless, it cannot be concluded that the size is, as well, influenced by milling ball size, ball material, etc. Thus, the author recommends studying other effects on size of the compound for the purpose of generating guided milling conditions to produce desired particle size. As there are many shapes of obtained ZnO, when the size, especially in rod shape, is reported, the author is suggested that length of the particle should be measured and added to the result in addition to its width. For more convenient and acceptable way of size interpretation, the author advises that a particle size analyzer be applied[22]. This will improve the accuracy level of size distribution as crystallite size, not agglomerated particle size, is appraised. Finally, a change in color of ZnO from white to soft yellow is noticed together with an increase in surrounding temperature after milled. It is discussed that whether hydrogen incorporation, oxygen deprivation, or a combination of multiple factors is the cause of this thermo-chromic phenomenon of ZnO[23].

According to the evaluation of antibacterial activity against *S. mutans*, the compound at all sizes exhibits the activity. This is especially the greatest for ZnO powder with the smallest size compared (S_1), revealing that its optimal MIC is equal to 28.125 μg/ml. The MIC results of these powders correlate with those present in other literatures, which the value ranges from 0.156 to 806.18 μg/m[9,10]. However, the process of antibacterial testing used in those studies is completely not the same as the one used in this study, and minimal bactericidal concentration (MBC) is suggested to be incorporated. More replications as well as controls should be performed to acquire a more dependable result. Additionally, it is still dubious about how MIC is interpreted if there is no clear inoculated well present, as ZnO and the inoculum both can make the medium turbid. Another recommendation for this part of experiment is to apply CAMHB with supplemental 2 – 5% v/v LHB following the suggested protocol instead of MHB used here; yet, there some reasons why MHB is used as medium of choice for susceptibility testing. To begin with, it shows acceptable batch-to-batch reproducibility for susceptibility testing. In addition, not only does it support satisfactory growth of most pathogens, it is also low in inhibitors that affect the results of some drugs. In the last place, a large body of data and experience has been gathered about tests performed with this medium[16].

Further study in this field of research is suggested on in-vivo antibacterial testing or testing of a change in properties after ZnO NP synthesized from brass ash is integrated into dental products or some other medical applications as additional features of nanoparticles have been widely reported due to their larger surface area to volume ratio[5,8]. Besides, even if antibacterial activity of ZnO against various strains of bacteria is reported, the test of these brass-ash derivatives against other bacteria, except *S. mutans*, is somehow recommended[9-11].

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