รายงานวิจัยฉบับสมบูรณ์

วัสดุผสมซิลค์ไฟโบรอิน/เจลาตินที่มียา gentamicin sulfate สำหรับประยุกต์ใช้เป็นวัสดุทางการแพทย์

Silk fibroin/gelatin blend scaffolds containing gentamicin sulfate for biomedical applications

โดย

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งานวิจัยนี้ได้รับเงินอุดหนุนการวิจัยจากมหาวิทยาลัยแม่ฟ้าหลวง

ประจำปีงบประมาณ พ.ศ.2555

กิตติกรรมประกาศ (Acknowledgement)

คณะวิจัยขอขอบคุณทุนอุดหนุนการวิจัย จากมหาวิทยาลัยแม่ฟ้าหลวงประจำปีงบประมาณ 2555 และขอขอบคุณศูนย์เครื่องมือและเทคโนโลยี มหาวิทยาลัยแม่ฟ้าหลวง และมหาวิทยาลัยแม่ฟ้าหลวงที่ให้ความอนุเคราะห์ในการใช้สถานที่ และเครื่องมือในการทำวิจัยจนสำเร็จลุล่วงด้วยดี

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หัวหน้าโครงการวิจัย
วันที่ 20 เดือน มกราคม พ.ศ. 2557

บทสรุปผู้บริหาร

เงินอุดหนุนโครงการวิจัย จากมหาวิทยาลัยแม่ฟ้าหลวง

ประจำปีงบประมาณ พ.ศ. 2555

ตั้งแต่เดือน 18 เมษายน พ.ศ. 2555 ถึง เดือน 17 เมษายน พ.ศ. 2556

1. ชื่อโครงการ (ภาษาไทย) วัสดุผสมซิลค์ไฟโบรอิน/เจลาตินที่มียา gentamicin sulfate สำหรับประยุกต์ใช้เป็นวัสดุทางการแพทย์

ชื่อโครงการ (ภาษาอังกฤษ) Silk fibroin/gelatin blend scaffolds containing gentamicin sulfate for biomedical applications

2. ชื่อหัวหน้าโครงการ/ สำนักวิชา/ สัดส่วนการทำวิจัย

2.1 หัวหน้าโครงการ

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สัดส่วนที่ทำการวิจัย 80%

2.2 ผู้ร่วมโครงการ

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3. ความสำคัญของปัญหาในการวิจัย

ในปัจจุบัน ประเทศไทยต้องนำเข้าวัสดุทางการแพทย์จากต่างประเทศ เพื่อใช้ตอบสนองความต้องการของประชาชนในประเทศ โดยต้องเสียค่าใช้จ่ายในการนำเข้าวัสดุทางการแพทย์ในราคาที่สูง ทำให้ไม่สามารถตอบสนองความต้องการของประชาชนที่มีรายได้ต่ำ ดังนั้นจึงมีความสนใจที่จะวิจัยพัฒนาวัสดุทางการแพทย์เพื่อทดแทน และลดการนำเข้าวัสดุทางการแพทย์จากต่างประเทศ โดยในงานวิจัยนี้ได้เลือกใช้พอลิเมอร์ 2 ชนิด คือ gelatin และ silk fibroin สำหรับใช้ขึ้นรูปเป็นวัสดุทางการแพทย์ เนื่องจากพอลิเมอร์ทั้ง 2 ชนิดนี้ ได้มาจากธรรมชาติ จึงมีคุณสมบัติที่ดี คือ ไม่เป็นพิษต่อเซลล์เนื้อเยื่อ ย่อยสลายได้เองตามธรรมชาติ เข้ากันได้กับเนื้อเยื่อ

สามารถดูดซับน้ำได้ดี อีกทั้ง silk fibroin ที่นำมาใช้ในงานวิจัยนี้ สามารถหาได้ง่ายในประเทศไทย โดยได้จากการสกัดรังไหมซึ่งพบได้ในประเทศไทยโดยเฉพาะแถบภาคอีสานและภาคเหนือ และยังมีฤทธิ์ช่วยสมานแผลได้อีกด้วย นอกจากนี้ gelatin ที่ใช้ในเป็นวัสดุในการขึ้นรูปยังมีราคาถูกโดยมีสมบัติเทียบเท่าคอลลาเจนซึ่งมีราคาสูง จากที่กล่าวมาแล้ว ในงานวิจัยนี้ยังได้มีการใส่ยา gentamicin sulfate ซึ่งมีฤทธิ์ต้านแบคทีเรียลงไปในวัสดุผสม เพื่อศึกษาการปลดปล่อยยา gentamicin sulfate จากวัสดุผสมที่ได้ และศึกษาความสามารถในการนำไปประยุกต์ใช้เป็นวัสดุทางการแพทย์ โดยทดสอบฤทธิ์ต้านแบคทีเรีย และความเป็นพิษของวัสดุผสมที่มียา gentamicin sulfate ก่อนที่จะนำไปประยุกต์ใช้จริงกับมนุษย์ ดังนั้นถ้าผลงานวิจัยนี้มีความสำเร็จดังที่ผู้วิจัยมุ่งหวังไว้ ก็จะส่งผลให้สามารถนำงานวิจัยไปต่อยอดเพื่อให้ได้วัสดุทางการแพทย์ที่เหมาะสมสำหรับรักษาผู้ป่วย มีราคาถูก และจะเป็นการช่วยเพิ่มทางเลือกให้มากขึ้น อีกทั้งยังสามารถลดการนำเข้าวัสดุทางการแพทย์จากต่างประเทศได้ นอกจากนี้อาจจะนำองค์ความรู้ที่เกิดขึ้น ไปพัฒนาต่อยอดวัสดุทางการแพทย์จากวัสดุชนิดอื่น ๆ โดยอาจจะมีการผสมผสานเทคโนโลยีและภูมิปัญญาท้องถิ่น เช่น ใช้สมุนไพรไทยที่พบได้ในท้องถิ่นเป็นตัวยาสำหรับศึกษาฤทธิ์ต้านต่าง ๆ ซึ่งอาจก่อให้เกิดทางเลือกใหม่ ๆขึ้นมาได้อย่างไม่มีที่สิ้นสุด

4. วัตถุประสงค์ของโครงการวิจัย

4.1 เพื่อขึ้นรูปวัสดุผสม silk fibroin/gelatin

4.2 เพื่อศึกษาลักษณะการปลดปล่อยยา gentamicin sulfate จากวัสดุผสม silk fibroin/gelatin

4.3 เพื่อศึกษาฤทธิ์ต้านเชื้อแบคทีเรีย และความเป็นพิษของวัสดุผสม silk fibroin/gelatin ที่มียา gentamicin sulfate

5. ขอบเขตของโครงการวิจัย

5.1 สกัด silk fibroin จากรังไหม

5.2 ทดสอบค่าความเป็นพิษของยา gentamicin sulfate ที่ใช้ผสมในวัสดุ ด้วยวิธี cytotoxicity

5.3 ศึกษาอัตราส่วนผสม และตัวแปรต่าง ๆในการขึ้นรูปวัสดุผสม silk fibroin/gelatin

5.4 ขึ้นรูปวัสดุผสม silk fibroin/gelatin ที่มียา gentamicin sulfate ผสมอยู่ ด้วยวิธี freeze drying

5.5 ศึกษาลักษณะสัณฐานวิทยา ลักษณะรูพรุน ของวัสดุผสมที่ขึ้นรูปได้

5.6 ศึกษาปริมาณการบวมตัว (Water swelling) และปริมาณการหายไปของน้ำหนัก (Weight loss) ของวัสดุผสมหลังจากแช่ในสารละลายบัฟเฟอร์ (Phosphate buffer solution)

5.7 ศึกษาลักษณะการปลดปล่อยยา gentamicin sulfate (Release characteristics) จากวัสดุผสม หลังจากแช่ในสารละลายบัฟเฟอร์ (Phosphate buffer solution) ที่เวลาต่าง ๆ

5.8 ศึกษาฤทธิ์ต้านแบคทีเรียของวัสดุที่มียา gentamicin sulfate และศึกษาความพิษของวัสดุที่มียา gentamicin sulfate ต่อเซลล์ผิวหนัง (human dermal skin fibroblast) ของมนุษย์ ด้วยวิธี indirect cytotoxicity

6. ผลผลิตจากการวิจัย

วัสดุผสม silk fibroin/gelatin ที่ขึ้นรูปได้นี้ เป็นวัสดุนำส่งยา gentamicin sulfate เพื่อศึกษาลักษณะการปลดปล่อยยาจากวัสดุผสม ฤทธิ์ต้านเชื้อแบคทีเรีย และความเป็นพิษของวัสดุผสม ซึ่งวัสดุผสมที่ได้จากงานวิจัยนี้ สามารถนำไปใช้เป็นวัสดุทางการแพทย์ โดยเฉพาะอย่างยิ่งวัสดุปิดแผล เนื่องจากยาปฏิชีวนะ gentamicin sulphate ที่ใช้ในวัสดุผสมนี้มีฤทธิ์ต้านเชื้อแบคทีเรียชนิด Gram-negative เช่น ใช้รักษาโรคติดเชื้อที่เกิดจาก pseudomonas, proteus, klebsiella, enterobacter จากการทดลองพบว่า วัสดุผสมที่ขึ้นรูปได้สามารถเป็นวัสดุนำส่งยา gentamicin sulfate ได้ และเมื่อทำการทดสอบฤทธิ์ต้านเชื้อแบคทีเรียของวัสดุผสมนี้ พบว่าวัสดุผสมมีความสามารถในการยับยั้งเชื้อแบคทีเรียได้ดี นอกจากนี้ เมื่อทำการทดสอบความเป็นพิษของวัสดุผสมนี้ พบว่า วัสดุผสมไม่มีความเป็นพิษต่อเซลล์ผิวหนัง ดังนั้นวัสดุผสม silk fibroin/gelatin ที่มียา gentamicin sulfate เหมาะสำหรับนำไปประยุกต์ใช้เป็นวัสดุปิดแผล เพื่อทดแทนวัสดุทางการแพทย์ที่นำเข้าจากต่างประเทศบางชนิดได้

7. ประโยชน์ที่ได้รับจากโครงการวิจัย ผลกระทบทางวิชาการหรือเทคโนโลยี

จากงานวิจัยนี้ ได้องค์ความรู้ใหม่ ซึ่งสามารถนำความรู้ที่ได้ไปต่อยอดโดยร่วมมือกับภาครัฐและเอกชนเพื่อผลักดันผลงานวิจัยเข้าสู่กระบวนการเชิงพาณิชย์ โดยผลิตเป็นวัสดุวัสดุปิดแผลเพื่อรักษาแผลของผิวหนังที่อาจจะเกิดจากการติดเชื้อของแบคทีเรีย ทั้งนี้เพื่อลดต้นทุนการผลิตและช่วยส่งเสริมคุณภาพชีวิตของผู้ป่วยให้ดีขึ้น

Abstract

Both the neat and the gentamicin sulfate (GS)-loaded silk fibroin (SF)/gelatin (Gel) blend scaffolds were fabricated by freeze drying method. The SF/Gel scaffolds were prepared by various blending ratio of SF and Gel solutions (i.e., 0/100, 30/70, 50/50, 70/30 and 100/0). 0.005 $mg \cdot mL^{-1}$ of GS powder was then added to prepare the GS-loaded SF/Gel blend scaffolds. These scaffolds were characterized for their morphology, pore size, mechanical property, water swelling and weight loss. The release characteristics of GS from the GS-loaded SF/Gel blend scaffolds were carried out in phosphate buffer solution. The antibacterial activity and the indirect cytotoxicity of these scaffolds were also investigated. From the results, the interconnected porous structure of these scaffolds was obtained. The pore size of the neat and the GS-loaded SF/Gel blend scaffolds ranged between 60 and 138 μm. Increasing SF content and addition of GS in scaffolds caused the compressive modulus of the scaffolds to decrease. Moreover, the water swelling and weight loss behaviors of these scaffolds increased with increasing submersion time. The cumulative amount of GS released from the GS-loaded SF/Gel blend scaffolds decreased with an increase of SF content in scaffolds. All scaffolds showed high activity against the growth of *S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus,* and *P. aeruginosa*. Lastly, all the GS-loaded SF/Gel blend scaffolds were proven non-toxic to NHDF cells except for the GS-loaded SF/Gel blend scaffolds at blending ratio of 100/0.

Key words: Silk Fibroin, Gelatin, Scaffolds, Gentamicin Sulfate, Wound Dressings

บทคัดย่อ

วัสดุผสมซิลค์ไฟโบรอิน/เจลาตินที่มี และไม่มียาเจนตามิซินซัลเฟต ถูกเตรียมในอัตราส่วนผสมของซิลค์ไฟโบรินและเจลาตินในอัตราส่วนต่างๆ คือ 0/100, 30/70, 50/50 และ 0/100 ด้วยวิธี freeze drying ยาเจนตามิซินซัลเฟตความเข้มข้น 0.005 มิลลิกรัม/มิลลิลิตรถูกใส่ไปในวัสดุผสมเพื่อเตรียมวัสดุผสมซิลค์ไฟโบรอิน/เจลาตินที่มียาเจนตามิซินซัลเฟต จากนั้นวัสดุผสมถูกนำไปศึกษาลักษณะสัณฐานวิทยา ขนาดของรูพรุน สมบัติเชิงกล การบวมตัวของน้ำและการสูญเสียน้ำหนัก นอกจากนี้ยังศึกษาลักษณะการปลดปล่อยยาเจนตามิซินซัลเฟต จากวัสดุผสมในสารละลายฟอสเฟต บัฟเฟอร์ ฤทธิ์การยับยั้งเชื้อแบคทีเรีย และความเป็นพิษของวัสดุผสมที่ขึ้นรูปได้ จากการผลการทดลองพบว่า วัสดุผสมที่ขึ้นได้มีลักษณะโครงสร้างรูพรุนเชื่อมต่อกัน มีขนาดรูพรุนอยู่ระหว่าง 60 ถึง 138 ไมโครเมตร ปริมาณซิลค์ไฟโบรอินที่เพิ่มขึ้นและการเติมยาเจนตามิซินซัลเฟตในวัสดุผสมส่งผลให้ค่ามอดุลัสแรงกดมีค่าลดลง นอกจากนี้การบวมตัวของน้ำและการสูญเสียน้ำหนักมีค่าเพิ่มขึ้นเมื่อแช่ในสารละลายฟอสเฟต บัฟเฟอร์นานขึ้น ยาเจนตามิซินซัลเฟตที่ปลดปล่อยออกมาจากวัสดุผสมมีค่าลดลงเมื่อปริมาณซิลค์ไฟโบรอินในวัสดุผสมเพิ่มขึ้น นอกจากนี้วัสดุผสมที่ขึ้นรูปได้มีความสามารถยับยั้งเชื้อแบคทีเรียที่พบในผิวหนังได้ เช่น *S. aureus S. epidermidis M. luteus B. cereus* และ *P. aeruginosa* สุดท้ายนี้พบว่าวัสดุผสมซิลค์ไฟโบรอิน/เจลาตินที่มียาเจนตามิซินซัลเฟตไม่เป็นพิษต่อเซลล์ผิวหนังของมนุษย์ ยกเว้นวัสดุผสมที่มีอัตราส่วนผสมของซิลค์ไฟโบรินและเจลาตินในอัตราส่วน 100/0

คำสำคัญ: ซิลค์ไฟโบรอิน เจลาติน วัสดุโครงร่าง เจนตามิซินซัลเฟต วัสดุปิดแผล

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CHAPTER 1
INTRODUCTION

1.1 Statement and significance of the problem

At present, Thailand has to import medical materials from abroad for the needs of people in the country. Since the cost of the importing medical materials is high, then this can not meet the needs of people who have low income. Thus, many researchers are interested to develop the medical materials or alternative materials to reduce the import of medical materials from abroad. In this research, silk fibroin (SF) and gelatin (Gel) are used to fabricate the medical materials. Since these polymers have several properties such as non-toxic to human cells, biodegradable, biocompatible, and good water absorption. Moreover, SF is available in Thailand, especially the eastern and northern parts of Thailand, and also has healing properties for wound. In addition, Gel has low cost and the equivalent properties of collagen. The addition of gentamicin sulfate (GS) which has the antibacterial properties in these materials was investigated to study the release characteristics of GS from these materials. Moreover, the cytotoxicity of these materials was investigated. If this research is successfully as researcher expected, it can lead to further research in order to obtain the low cost and suitable medical materials for patient. It can also reduce the import of the medical materials from abroad. Moreover, it can further develop medical materials from other materials and may be a combination of technology and traditional knowledge, such as Thai herbs, which may cause the new knowledge and infinite development.

1.2 Objectives

1. To fabricate GS-loaded SF/Gel blend scaffolds
2. To study the release characteristics of GS from GS-loaded SF/Gel blend scaffolds
3. To study the antibacterial activity and cytotoxicity of GS-loaded SF/Gel blend scaffolds

CHAPTER 2
LITERATURE REVIEWS

Scaffold is a one type of wound dressing that is a substrate for the implanted cells and a physical support to control the restoration of a tissue or replacing an organ (Parveen, *et al.*, 2006). In addition to facilitating cell attachment, promoting cell growth, and allowing the retention of differentiated cell functions, the scaffold should be biocompatible, biodegradable, mechanically strength, malleable, and highly porous with a large surface/volume ratio (Chen, *et al.*, 2002). Various natural and synthetic polymers can be used to produce the scaffolds. Natural polymers have been widely used for biomedical applications including collagen, fibroin, gelatin, chitosan, alginate, and hyaluronic acid (Adekogbe, *et al.*, 2005; Woei, *et al.*, 2001). While synthetic polymers also have been widely used such as polycaprolactone (PCL), polylactic acid (PLA), polyglycolic acid (PGA), and poly(lactide-co-glycolide) (PLGA). Moreover, several processing techniques have been developed to fabricate polymeric scaffolds including fiber bonding, electrospinning, solvent casting/particulate leaching, gas foaming/high pressure processing, and freeze drying (Gunatillake, *et al.*, 2003).

Freeze drying is based on the formation of ice crystals that induce porosity through ice sublimation and desorption. The kinetic of the freezing stage controls the porosity and the interconnectivity of scaffolds by removing water or solvent from emulsion to yield the highly inter-connected pores of scaffolds (Liapis, *et al.*, 1996). This process can prepare the porosity up to 90%, and diameter of scaffolds in a range of 15-35 μm. Advantages of this process are highly porous structure and highly pore interconnectivity. But it is limited to small pore size (Whang, *et al.*, 1995). In 1999, Kang, *et al.* studied the fabrication of porous gelatin scaffolds by using water as a porogen and freeze drying technique. The porous structure of scaffolds can be controlled by varying the freeze drying condition. The fast freezing process produced the scaffolds with small pore size, while large pore size of the scaffolds was obtained from the slow freezing process.

Controlled release systems are used to improve therapeutic efficiency and safety of drugs by delivering them a rate dictated by the need of the physiological environment over a period of treatment to the site of the action (Kenawy, *et al.,* 2002). The aims of controlled drug release are improving the effectiveness of drug therapy, increasing therapeutic activity compared to the intensity of side effect, reducing the number of drug administration required during treatment, or eliminating the need for specialized drug administration. Human skin consists of two distinct layers: the stratified vascular cellular epidermis and an underlying dermis of connective tissue. A fatty subcutaneous layer resides beneath the dermis. Hairy skin develops hair follicles and sebaceous glands, and the highly vascularized dermis supports the apocrine and eccrine sweat glands, which pass through pores in the epidermis to reach the skin surface (Barry, *et al.,* 2004). With respect to drug permeation, the most important component in this complex membrane is the stratum corneum, or horny layer, which usually provides the rate-limiting or slowest step in the penetration process.

The mechanisms of drug transportation by crossing the intact skin have not yet been completely elucidated. However, possible macro-routes may comprise the transdermal pathway or via the hair follicles and sweat glands. The appendageal route may be significance for short diffusion times and for polar molecules. Until recently, it was believed that, for polar molecules, the probable route was via the hydrated keratin of the corneocyte. However, it now seems more probable that the dominant pathway is via the polar region of intercellular lipid, with the lipid chains providing the nonpolar routes shown in Figure 2.1 (Langer, *et al.*, 2004).

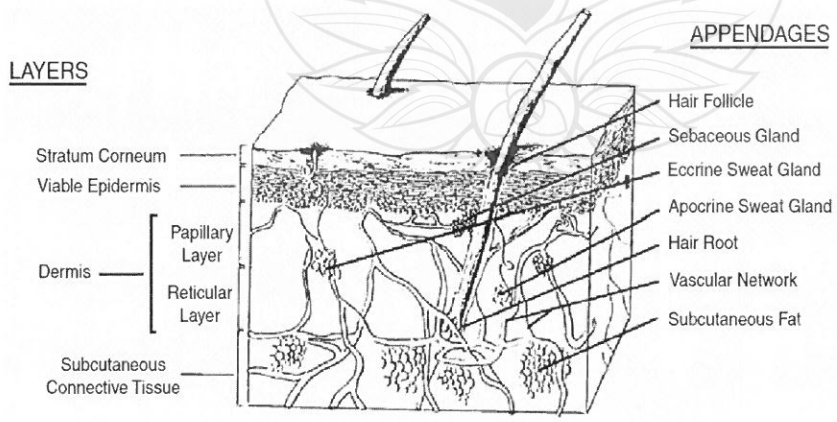


Figure 2.1 Basic diagram of skin structure.

In 1984, Wise, *et al.* reported that the relative importance of these routes depends upon numerous factors, such as the time-scale of permeation, the physicochemical properties of the penetrant (e.g., pKa, molecular size, stability, binding affinity, solubility, and partition coefficient), integrity and thickness of the stratum corneum, density of sweat glands and follicles, skin hydration, metabolism, and vehicle effects.

Most modern dressings are made from polymers which can serve as carriers for the delivery of drugs to wound sites. The polymeric dressings have been employed for controlled drug delivery to wounds including hydrogels such as poly(lactide-co-glycolide), poly(vinyl pyrrolidone), poly(vinyl alcohol) and poly(hydroxyalkylmethacrylates), polyurethane-foam, hydrocolloid and alginate dressings. Other polymeric dressings reported for drug delivery to wounds comprise novel formulations prepared from polymeric biomaterials such as hyaluronic acid, collagen, and chitosan. Synthetic polymers have been employed as swellable dressings for controlled drug delivery including silicone gel sheets and polylactic acid (Boateng, *et al.*, 2007). Composite dressings comprising both synthetic and naturally occurring polymers have also been reported for controlled drug delivery to wound sites (Sakchai, *et al.*, 2006). The modern dressings for drug delivery to wounds may be applied in the form of gels, films and foams, while, the novel polymeric dressings produced in the form of films and porous sponges such as freeze-dried wafers or discs or as tissue engineered polymeric scaffolds (Kumar, *et al.*, 2004). Zhang, *et al.*, (2002) prepared macroporous chitosan scaffolds reinforced by calcium phosphates (CaP) particles such as β-tricalcium phosphate (β-TCP) and CaP invert glasses using a thermally induced phase-separation technique. These porous composite materials were loaded with gentamicin sulfate (GS) by immersing them in GS-containing PBS solutions. The results showed that in comparison with GS-loaded pure chitosan scaffolds, the initial high burst release of GS was decreased through incorporating CaP crystals and glass particles into the scaffolds, and the sustained release for more than three weeks was achieved. The highest sustained release was observed from the particle-containing composite, which was suggested to occur owing to a higher extent of chitosan cross-linking. The cells attached and migrated on these scaffolds, suggested that these scaffolds are good cellular compatibility (Habraken, *et al.*, 2007).

A new design of a tissue engineering (TE) scaffold with controlled drug-delivery capability has been developed by Shi, *et al.*, (2009). The scaffold is based on mesoporous silica HA (HMS HA) composite particles used as fillers in poly(lactic-co-glycolic acid) or PLGA microspheres. HMS HA particles were produced using dodecylamine as a template and GS-loaded PLGA microspheres were prepared using a double emulsion solvent evaporation technique (water/oil/water). PLGA/HMS HA GS composite microspheres were prepared using a single emulsion solvent evaporation method. PLGA or PLGA/HMS HA GS microsphere sintered scaffolds were subsequently fabricated by pouring PLGA or PLGA/HMS HA GS microspheres into cylindrical moulds, and subsequently sintering at 70 °C for 2 h. The results showed that the presence of HA in PLGA/HMS HA scaffolds could balance the decreased pH values caused by the acidic degradation product of PLGA. Moreover, HMS HA improved the cytocompatibility and bioactivity of PLGA. It was also claimed that the compressive strength and elastic modulus of PLGA/HMS HA scaffolds were higher than those of pure PLGA scaffolds, showing similar mechanical properties to human cancellous bone. In vitro drug-delivery testing in the simulated body fluid (SBF) of the PLGA/HMS HA scaffolds showed that PLGA reduced the GS release from HMS HA particles, and the release lasted for nearly one month.

Silk is a natural polymer produced by the silk worm and their major components are fibroin and sericin that are a protein. Silk fibroin (SF) consists of heavy (350 kDa) and light (25 kDa). In the heavy chains compose of glycine, alamine and sericin residues, which can be formed into β-sheet crystalline structure. SF can be used in biotechnology and biomedical materials because it has various properties such as biocompatibility, mechanical strength, high thermal stability, microbial resistance, and biodegradability properties (Gil, *et al.*, 2007).

Gelatin (Gel) is a natural polymer denatured form of collagen and contains a number of functional groups such as amino acids that found in animal tissue. Collagen is the major protein component of extracellular matrices in animal (ECM) (Sai, *et al.*, 2000). Collagen has highly antigenicity due to its animal origin but gelatin has relatively low-antigenic and lower cost (Lien, *et al.*, 2008). This material has the limitation of low mechanical strength and is effectively used only as incorporated

component with others polymers to modify the biological or mechanical properties (Ding, *et al.*, 2005). Gelatin has been blended with other organic or inorganic biomaterials to fabricate the dehydrate form such as 3-D scaffolds, hydrogels, and films. There are several properties of gelatin such as biological origin, biodegradability, and biocompatibility. Thus, it is suitable used for wound dressing, drug delivery system, and tissue engineering applications (Zhong, *et al.*, 2010).

Gentamicin sulfate (GS) is an aminoglycoside antibiotic for treating many types of bacterial infections that caused from gram-negative bacteria such as *Pseudomonas aeruginosa* (Lu, *et al.*, 1996). Suzuki, *et al.*, (1998) investigated releasing of gentamicin with *P. aeruginosa* that found in the infected wound, by blending gentamicin with polyvinyl alcohol derivative (PVA) to occur hydrogel. Gentamicin was release at specific times and location where *P. aeruginosa* infection occurs. This ability indicated the selective release of gentamicin in *P. aeruginosa*-infected wound fluid and important to reduce its growth in *vitro*. A recent research, GS-loaded into silk fibroin/elastin blends scaffolds were fabricated to treat burn wound as wound dressing. This study had shown that the releasing of GS increased with an increase in the elastin content because it was affected to higher pore size of scaffolds (Vasconcelos, *et al.*, 2012).

From these literature reviews, SF and Gel are suitable for using as medical materials. In 2007, Gil, *et al.* fabricated Gel/SF films by solvent casting and then treated these films with methanol to induce SF structure from amorphous to crystalline region resulting in the insoluble of Gel/SF films in water. Fan *et al.* have fabricated Gel/SF scaffolds for use in ligament tissue engineering. In this research, study the feasibility of using co-culture system to induce the differentiation of Mesenchymal stem cells (MSCs) for fabricating the tissue-engineered ligament in vitro. From results, the MSCs were distributed uniformly throughout these scaffolds and exhibited good cell viability. The MSCs in co-culture system can differentiate into ligament fibroblast. The effects of different freezing temperature on silk fibroin pore structure were investigated to study cell proliferation and migration (Mandal, *et al.*, 2009). The results showed that the rapid freeze drying method fabricated highly interconnected porous scaffolds for using in tissue engineering. The pore size, porosity and interconnectivity of scaffolds have affected to the cell proliferation and

migration on scaffolds. Mandal, *et al.* prepared multilayered films based on silk fibroin and gelatin for use in controlled drug release. The films were investigated for release using trypan blue, FITC-inulin and FITC-BSA as model drugs. The results showed that the release characteristic of compounds exhibited dependence on multilayer film degradation for sustained release. From results, the silk fibroin/gelatin multilayer films are good candidates fro the controlled release of a wide spectrum of bioactive molecules (Mandal, *et al.*, 2009). Moreover, Mandal, et al. fabricated novel 3-D sericin/gelatin scaffolds and 2-D films using non-mulberry Antheraea mylitta silk cocoon sericin protein. These materials were characterized and optimized for biomedical applications. The results showed that blended sericin/gelatin 3-D scaffolds were highly porous with an optimum pore size of 170 ± 20 μm. In addition, these materials enhanced cell attachment and proliferation making them suitable for use in tissue engineering applications (Mandal, *et al.*, 2009).

CHAPTER 3
METHODOLOGY

3.1 Materials

The raw silk cocoons of mulberry bombyx mori were obtained from Ubonratchathani province (Thailand). The type A gelatin (MW = 1,400,000 g/mol) from porcine skin and glutaraldehyde solution were purchased from Fluka Analytical (Switzerland). Gentamicin sulfate (GS) powder was obtained from Shijiazhuang Pharm-chem Technology (China).

3.2 Preparation of SF solution

Silk cocoons were cut into small pieces, boiled with Na_2CO_3 for 45 min twice to degumming and then washed it with deionized water in several time to remove sericin out. Degummed silk was dissolved in $CaCl_2$: EtOH: Water (1:2:8) solution at 85 °C for 3 hours. SF solution was obtained and dialyzed with deionized water for 2-3 days by changing deionized water every 6 h to remove solvent. SF solution was centrifuged at 5,000 rpm for 20 min at 10°C to remove lipid precipitate. Then, SF solution was lyophilized by using freeze drying method for 20 h. Fourier transform infrared spectroscopy (FTIR) analysis of the obtained silk fibroin was carried out using an FTIR spectrometer (Spectrum GX, Perkin-Elmer) in the spectral region of 4000 400 cm^{-1} with 4 cm^{-1} resolution.

3.3 Indirect cytotoxicity of GS

The human dermal fibroblast cells were grown in Dulbecco s Modified Eagle s Medium (DMEM) supplemented with 10% fetal bovine serum, 2mM L-glutamine, 100 unit/mL penicillin and 100 ug/mL streptomycin. The cells were incubated at 37 ºC in a fully humidified, 5% CO_2: air atmosphere.

GS was weighed and dissolved in sterile distilled water to make a stock concentration of 100 $mg{\cdot}mL^{-1}$, and then was serial diluted in the culture medium of

cells at a ratio of 1:2 giving 8 concentrations of 5000, 2500, 1250, 625, 312.5, 156.25, 78.125 and 39.06 $\mu g \cdot mL^{-1}$.

The MTT assay is a tetrazolium-dye based colorimetric microtitration assay. Metabolism competent cells are able to metabolize the tetrazolium (yellow) to formazan (blue); this color change is measured spectrophotometrically with a plate reader. It is assumed cells that are metabolically deficient will not survive, thus the MTT assay is also an indirect measurement of cell viability. The cells were seeded in a 96-well plate at a density of 5,000 cells/well, and incubated for 48 hours. The samples at various concentrations were added to the cells and incubated for 24 hours. The test samples were removed from the cell cultures and the cells were reincubated for a further 24 hours in fresh medium and then tested with MTT assay.

Briefly, 50 µl of MTT in PBS at 5 $mg \cdot mL^{-1}$ was added to the medium in each well and the cells were incubated for 4 hours. Medium and MTT were then aspirated from the wells, and formazan solubilized with 200 µL of DMSO and 25 µL of Sorensen s Glycine buffer, pH10.5. The optical density was read with a microplate reader (Molecular Devices) at a wavelength of 570 nm. The average of 4 wells was used to determine the mean of each point. The data were analyzed with the SoftMax Program (Molecular Devices) to determine the IC_{50} for each toxin sample. A dose-response curve was derived from 8 concentrations in the test range using 4 wells per concentration. Results of toxic compounds are expressed as the concentration of sample required to kill 50% (IC_{50}) of the cells compared to controls.

3.4 Preparation of neat and GS-loaded SF/Gel blend scaffolds

The SF/Gel blend scaffolds were fabricated by weight blending ratio of SF and Gel solution as 0/100, 30/70, 50/50, 70/30 and 100/0. The GS-loaded SF/Gel blend scaffolds were prepared by adding 0.005 $mg \cdot mL^{-1}$ GS powder into SF/Gel solutions. 0.75%v/v glutaraldehyde (GTA) solution was added to each blended solution to crosslink for 15 min at room temperature. These solutions were poured into polypropylene (PP) mold and freeze dried to form the GS-loaded SF/Gel blend scaffolds. Then, methanol (MeOH) treatment was used to induce the structure of silk fibroin to form β-sheet conformation by submerging these scaffolds into 80% MeOH solution for 30 min and then lyophilized again.

3.5 Morphological observation and pore size measurement

Morphological appearance of both the neat and the GS-loaded SF/Gel blend scaffolds was observed under a JEOL JSM-5410LV Scanning Electron Microscope (SEM). Each specimen was coated with a thin layer of gold using a JEOL JFC-1100E sputtering device prior to observation under SEM. Pore size of these scaffolds were measured directly from SEM images using SemAphore 4.0 software. These values were averaged to obtain the pore size of the particular pore. More than 30 pores for each sample group were measured

3.6 Mechanical test

The compressive modulus of both the neat and the GS-loaded SF/Gel blend scaffolds were characterized with Instron Machine Model 5566 universal testing machine using 1 kN load cell at room temperature. Both the neat and the GS-loaded SF/Gel blend scaffolds were compressed at the crosshead speed of 1 $mm \cdot min^{-1}$ until the samples were around 70% deformed from their original height of ~15 mm. The obtained data were modified by connecting with computer for control apparatus and analyzing of the results.

3.7 Water swelling and weight loss

The water swelling and the weight loss behaviors of the neat and the GS-loaded SF/Gel blend scaffolds were measured in a phosphate buffer solution at the physiological temperature of 37 ℃ for 24 and 48 h. The measurements of each sample were calculated according to the following equations:

$$\text{Water swelling}\,(\%) = \frac{M - M_{\mathrm{d}}}{M_{\mathrm{d}}} \times 100, \qquad (1)$$

And

$$\text{Weight loss}\,(\%) = \frac{M_{\mathrm{i}} - M_{\mathrm{d}}}{M_{\mathrm{i}}} \times 100, \qquad (2)$$

where M is the weight of each sample after submersion in the buffer solution for 24 and 48 h, M_d is the weight of each sample after submersion in the buffer solution for 24 and 48 h in its dry state, and M_i is the initial weight of each sample in its dry state.

3.8 Release of GS from GS-loaded SF/Gel blend scaffolds

3.8.1 Actual GS content

The actual amount of GS in the GS-loaded SF/Gel blend scaffolds was first determined. Each scaffold was immersed in 10 mL of phosphate buffer solution. Then 1 mL of the solution was determined using a Perkin-Elmer UV-Vis spectrophotometer at the wavelength of 570 nm. The actual amount of GS in the GS-loaded SF/Gel blend scaffolds was then back-calculated from the resulting data against a predetermined calibration curve.

3.8.2 GS release assay

The release characteristics of GS from the GS-loaded SF/Gel blend scaffolds were investigated by total immersion method in the phosphate buffer solution. Each scaffold was immersed in 10 mL of the phosphate buffer solution at 37 °C. After a specified immersion time ranging between 0 and 48 h (2880 min), 1 mL of sample solution was withdrawn and fresh medium was refilled. Since GS did not absorb ultraviolet or visible light, then the ninhydrin solution was used to mix with the sample solution. The amount of GS in sample solution was determined using a Perkin-Elmer UV-Vis spectrophotometer at the wavelength of 570 nm. The obtained data were calculated to determine the cumulative amount of GS released from the GS-loaded SF/Gel blend scaffolds.

3.9 Antibacterial evaluation of GS-loaded SF/Gel blend scaffolds

The AATCC Test Method 100 (Antibacterial Finishes on Textile Materials: Assessment of The American Association of Textile Chemists and Colorists) or Colonies count was used to investigated the antibacterial activity of the GS-loaded SF/Gel blend scaffolds. The pathogenic bacteria, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Bacillus cereus*, and *Pseudomonas*

aeruginosa, were used to investigate. First, 1.0 ml of culture medium (10^5 CFU/ml) was added into each sample and then kept it in incubator at 37 ºC for 24 h. For control, the neat SF/Gel blend scaffolds were tested. After 24 h incubation, the bacteria were eluted from the sample by adding 5 mL of sterilized deionized water into the sample with vigorously shaking for 5 minutes at room temperature. Then, the eluted solutions were made a series dilution by using 0.1% peptone. The series diluted solutions were spread (in triplicate) on nutrient agar (NA) plate. These plates were incubated at 37 °C for 24 h. Finally, the colonies on agar plate were photographed and counted (range of 30-300 colonies) to evaluate the antibacterial activity. The number of bacteria presented in the liquid was determined (on agar plate) and the percentage of reduction was also calculated.

The number of colony was counted as the number of bacteria per sample not as the number of bacteria per mL of neutralizing solution. The percent reduction of bacteria (R, %) was calculated by the following equation:

$$R\,(\%) = \frac{100\,(B - A)}{B} \qquad (3)$$

where A is the number of bacteria recovered from the incubated treated test sample (GS-loaded SF/Gel blend scaffolds) after 37 ºC for 24 h and B is the number of bacteria recovered from the incubated untreated test sample (neat SF/Gel blend scaffolds) after 37 °C for 24 h.

3.10 Indirect cytotoxicity evaluation of GS-loaded SF/Gel blend scaffolds

The indirect cytotoxicity evaluation of the GS-loaded SF/Gel scaffolds was conducted in 24-well tissue-culture polystyrene plate (TCPS; Corning Costar®, USA) using normal human dermal fibroblasts (NHDF; 13th passage). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, USA), containing 10% fetal bovine serum (FBS; Invitrogen Corp., USA), 1% L-glutamine (Invitrogen Corp., USA) and 1% antibiotic and antimycotic formulation [containing penicillin G sodium, streptomycin sulfate, and amphotericin B (Invitrogen Corp.,

USA)]. The samples cut from the GS-loaded SF/Gel blend scaffolds were first sterilized by UV radiation for ~1 h and then immersed in serum-free medium (SFM; containing DMEM, 1% L-glutamine and 1 % antibiotic and antimycotic formulation) for 24 h in incubation to produce extraction media. NHDF cells were separately cultured in wells of TCPS at 8,000 cells/well in serum-containing DMEM for 24 h to allow cell attachment. The cells were then starved with SFM for 12 h. After that, the medium was replaced with an extraction medium and the cells were re-incubated for 24 h. The viability of the cells cultured by each of the extraction media was finally determined with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The viability of the cells cultured by the fresh SFM was used as control.

The MTT assay is based on the reduction of the yellow tetrazolium salt to purple formazan crystals by dehydrogenase enzymes secreted from the mitochondria of metabolically active cells. The amount of the purple formazan crystals is proportional to the number of viable cells. First, the culture medium in each plate was aspirated and replaced with 25 μL/well of MTT solution at 5 mg mL^{-1}. The plate was further incubated for 4 h at 37 °C. The solution was then aspirated and 100 μL/well of dimethylsulfoxide (DMSO; Sigma-Aldrich, USA) was added to dissolve the formazan crystals. After 3 min of rotary agitation, the absorbance at the wavelength of 570 nm representing the viability of the cells was measured using a SpectraMax M2 Microplate Reader.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Preparation of SF solution

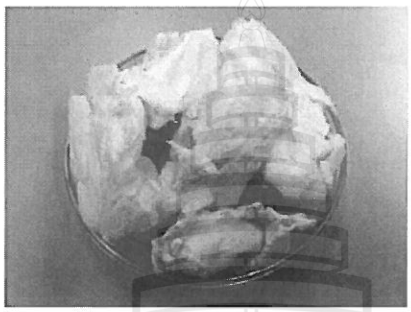


Figure 4.1 Silk fibroin (SF).

The SF samples were prepared as potassium bromide (KBr) pellets and characterized by using FTIR. Amide I and II showed the characteristic peaks of random coil and α-helix conformation at 1654.70 and 1541.03 cm^{-1}, respectively. Amide III and IV were also showed at absorption peak of 1242.02 and 1070.12 cm^{-1}, respectively, which more intense in $CaCl_2$:EtOH:H_2O protocol. Moreover, a broad peak around the wave number at upper than 3000 cm^{-1} is overlapped peak of NH and OH stretching (Figure 4.2).

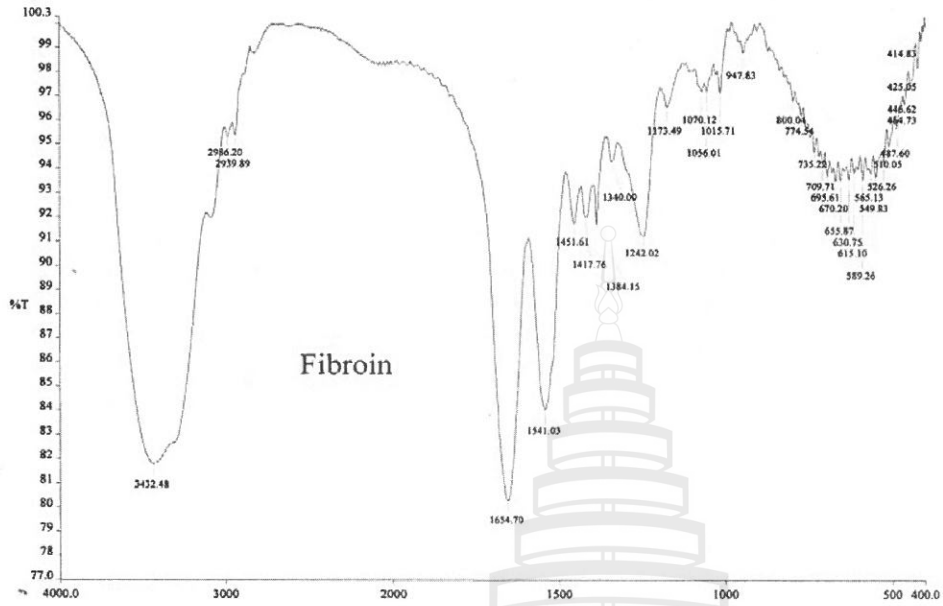


Figure 4.2 FTIR spectrum of SF.

4.2 Indirect cytotoxicity evaluation of GS

The % survival of the cells cultured with GS at various concentrations (compared to control) is summarized in Table 4.1.

Table 4.1 Indirect cytotoxicity of GS.

GS concentration ($\mu g \cdot mL^{-1}$)	% Cell survival
5000	68.47 ± 1.25
2500	82.02 ± 2.30
1250	88.36 ± 0.57
625	88.67 ± 0.70
312.5	93.76 ± 3.71
156.25	94.37 ± 1.21
78.125	99.20 ± 1.15
39.06	99.75 ± 1.04

The indication of toxicity has been evaluated in 2 ranges:

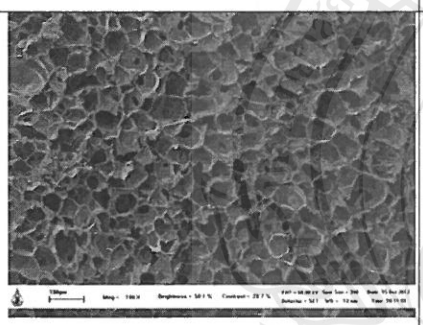
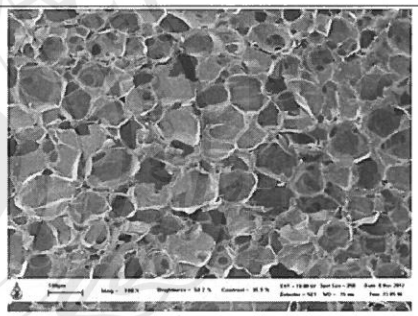
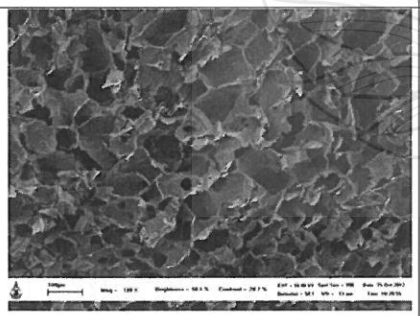
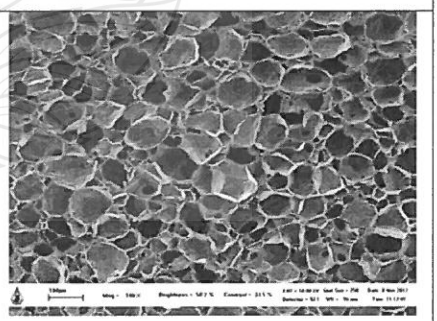
a) At %cell survival ≥ 50% will be evaluated for no toxicity

b) At %cell survival ≤ 50% will be evaluated for toxicity with IC_{50}

From Table 4.1, the IC_{50} of GS was more than 5,000 $\mu g \cdot mL^{-1}$. Thus, GS was non-toxic to human dermal fibroblast cell lines over the test concentration ranged up to 5,000 $\mu g \cdot mL^{-1}$.

4.3 Morphology and pore size of neat and GS-loaded SF/Gel blend scaffolds

Table 4.2 Selected SEM images of the neat and the GS-loaded SF/Gel blend scaffolds.

SF/Gel	SEM	GS-loaded SF/Gel	SEM
0/100		0/100	
30/70		30/70	

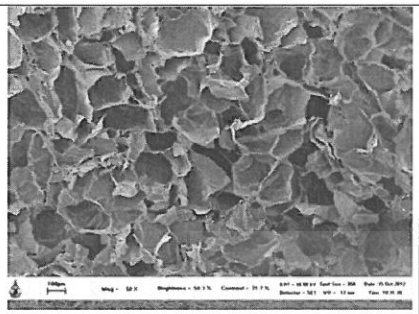
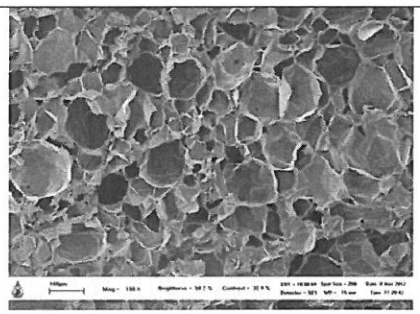
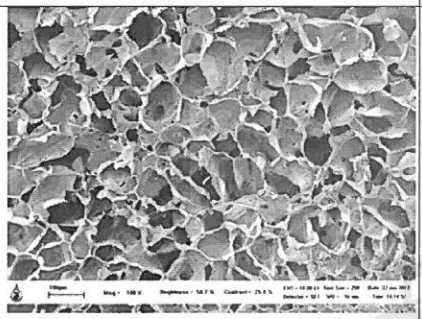
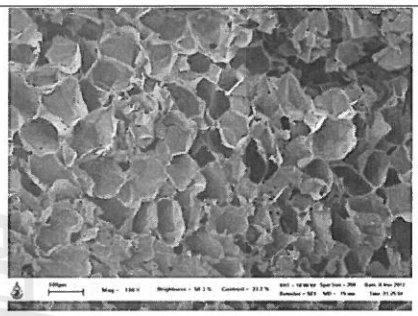
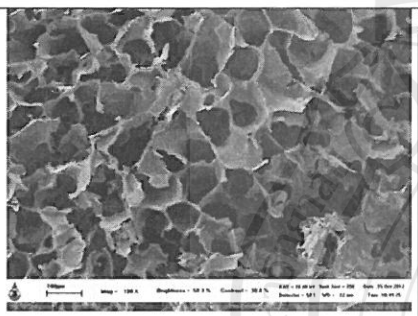
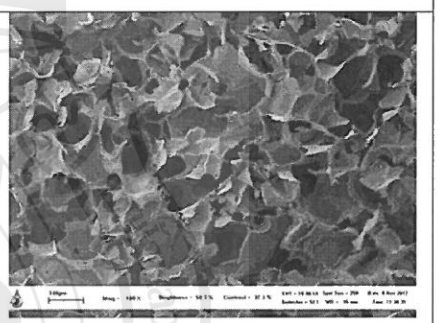
50/50		50/50	
70/30		70/30	
100/0		100/0	

Table 4.3 Pore sizes of the neat and the GS-loaded SF/Gel blend scaffolds.

SF/Gel	Pore sizes (μm)	GS-loaded SF/Gel	Pore sizes (μm)
0/100	60.51 ± 13.23	0/100	77.34 ± 23.12
30/70	68.51 ± 21.41	30/70	88.23 ± 26.97
50/50	138.18 ± 54.79	50/50	93.37 ± 27.60
70/30	84.10 ± 29.40	70/30	82.18 ± 25.89
100/0	103.87 ± 32.81	100/0	100.20 ± 27.83

4.4 Mechanical property

Desirable scaffolds should maintain a fixed shape and have enough mechanical strength in order to provide sufficient free space for cell attachment, proliferation and differentiation when cultured with cells *in vitro* or implanted *in vivo*. Compressive test is widely accepted to evaluate the mechanical strength of scaffolding materials. This property is important because it is closely associated with shape-retention ability in practical operations and applications. For this purpose, the compressive modulus of these scaffolds was investigated. The compressive modulus was calculated in the 2-6 %strain, since the extent is small in the first region of scaffolds compressive deformation that considered as elastic region (Zhou, *et al.*, 2005). The compressive modulus of both the neat and the GS-loaded SF/Gel blend scaffolds at the blending ratio of 0/100, 30/70, 50/50, 70/30 and 100/0 is shown in Figure 4.3. The compressive modulus of the neat SF/Gel blend scaffolds at the blending ratio of 0/100, 30/70, 50/50, 70/30 and 100/0 was ~0.81, ~0.75, ~0.70, ~0.44, and ~0.15 MPa, respectively. While the compressive modulus of the GS-loaded SF/Gel blend scaffolds at the blending ratio of 0/100, 30/70, 50/50, 70/30 and 100/0 was decreased to ~0.58, ~0.39, ~0.21, ~0.16, and ~0.12 MPa, respectively. From these results, increasing SF content decreased the compressive modulus of scaffolds. In addition, the addition of GS into scaffolds caused the compressive modulus of scaffolds to decrease. These results could be explained from Table 4.2. Since the neat and the GS-loaded Gel scaffolds had small pore size and good arrangement of porous structure so this might affect the better dispersion of force applying into samples. Moreover, the neat and the GS-loaded SF scaffolds showed non-uniform porous structure that caused the compressive modulus to decrease. Thus, it could be concluded that the pore size and the distribution of porous structure might affect the mechanical properties of these scaffolds.

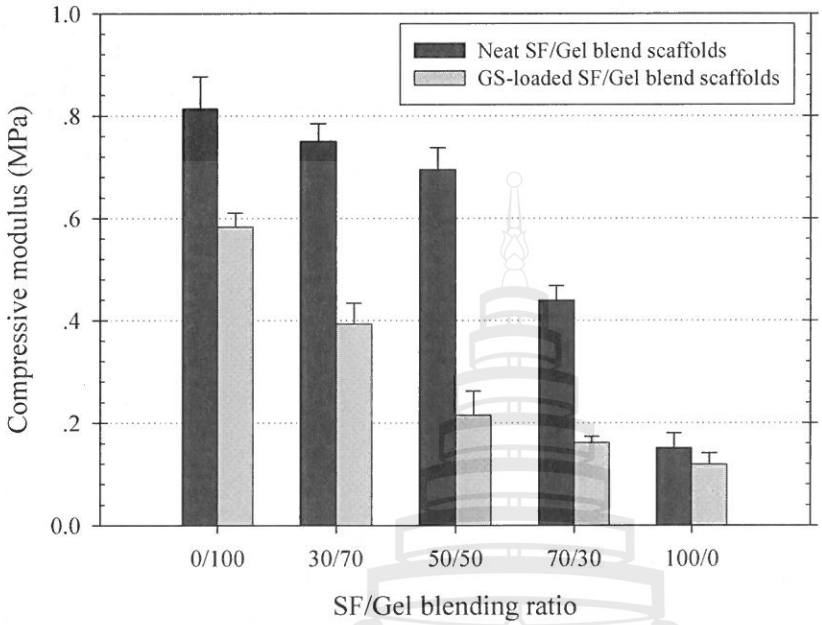


Figure 4.3 Compressive modulus of the neat and the GS-loaded SF/Gel blend scaffolds at the blending ratio of 0/100, 70/30, 50/50, 30/70, and 100/0 (n = 5).

4.5 Water swell and weight loss

The ability of a scaffold to absorb and retain exudates in its pore channels is very important property of a functional scaffold in actual use, as exudates contain cells and various biological entities which are essential for tissue regeneration within the scaffolds. The water swelling and the weight loss behaviors of both the neat and the GS-loaded SF/Gel blend scaffolds after submersion in phosphate buffer solution for 24 and 48 h at 37 °C were investigated and the results are shown in Figure 4.4 and 4.5. The water swelling of the neat and the GS-loaded SF/Gel blend scaffolds is shown in Figure 4.4. The water swelling of the neat SF/Gel blend scaffolds at the blending ratio of 0/100, 30/70, 50/50, 70/30 and 100/0 after submersion for 24 h was ~944, ~1265, ~960, ~1068 and ~1076%, respectively. At 48 h, the value increased to ~1183, ~1342, ~1217, ~1400 and ~1192%, respectively. While the water swelling of the GS-loaded SF/Gel blend scaffold at the blending ratio of 0/100, 30/70, 50/50, 70/30 and 100/0 after submersion for 24 h was ~1133, ~1035, ~1199, ~1279 and

~1106%, respectively. At 48 h, the value increased to ~1347, ~1610, ~1295, ~1513 and ~1218%, respectively.

The weight loss of the neat and the GS-loaded SF/Gel blend scaffolds is shown in Figure 4.5. The weight loss of the neat SF/Gel blend scaffolds at the blending ratio of 0/100, 30/70, 50/50, 70/30 and 100/0 after submersion for 24 h was ~11, ~12, ~7, ~2 and ~2%, respectively. At 48 h, the value increased to ~53, ~30, ~12, ~7 and ~5%, respectively. While the weight loss of the GS-loaded SF/Gel blend scaffold at the blending ratio of 0/100, 30/70, 50/50, 70/30 and 100/0 after submersion for 24 h was ~14, ~12, ~8, ~9 and ~4, respectively. At 48 h, the value increased to ~85, ~56, ~25, ~15 and ~10%, respectively.

From these results, both the water swelling and the weight loss increased with increasing submersion time. The increase in the submersion time caused the more water molecule to diffuse into the scaffolds leading to increase both the water swelling and the weight loss. Moreover, the addition of GS into scaffolds caused both the water swelling and the weight loss to increase. Because GS molecules which are good water soluble are highly diffuse into phosphate buffer solution.

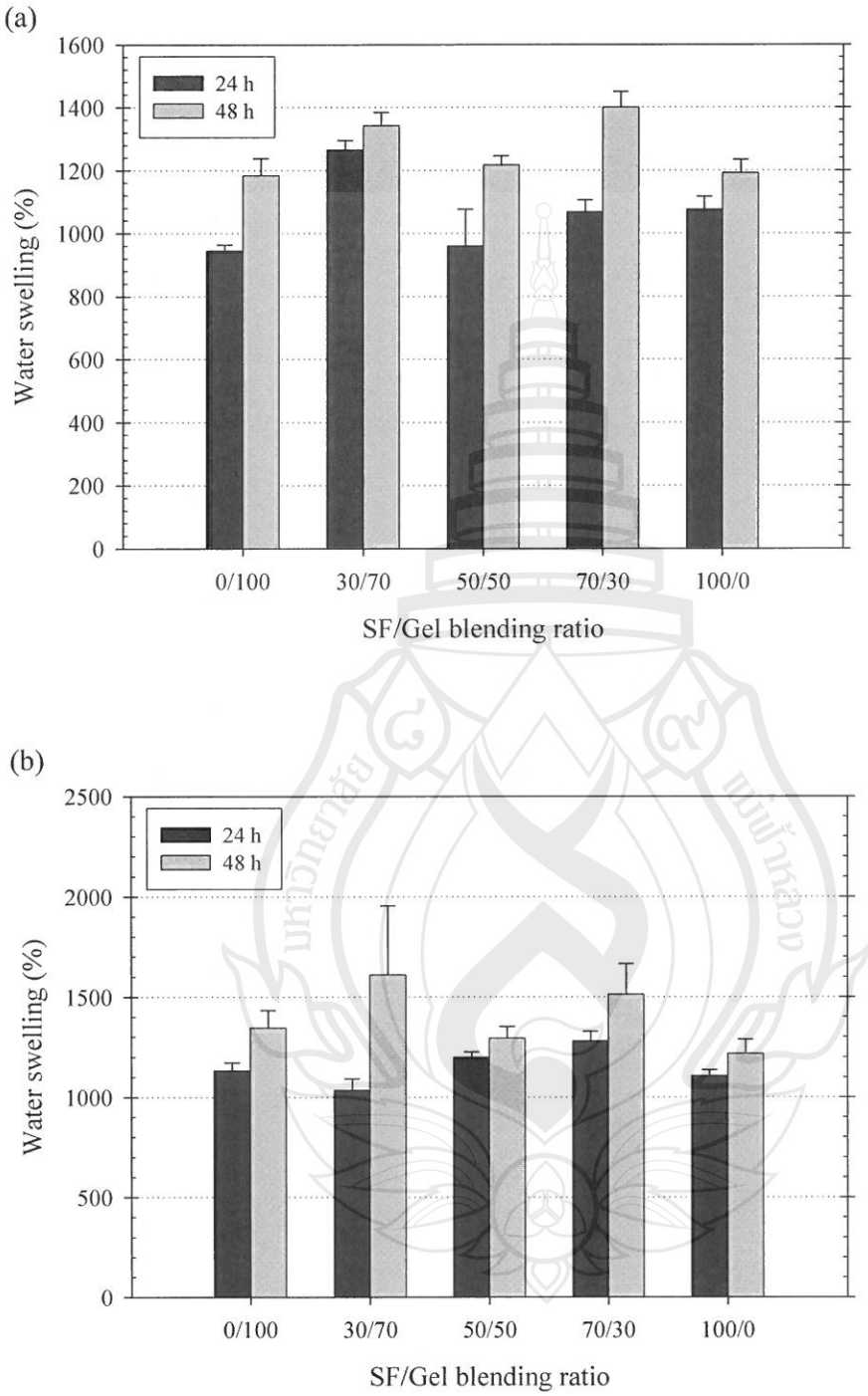


Figure 4.4 Water swelling of (a) the neat and (b) the GS-loaded SF/Gel blend scaffolds in phosphate buffer solution for 24 and 48 h.

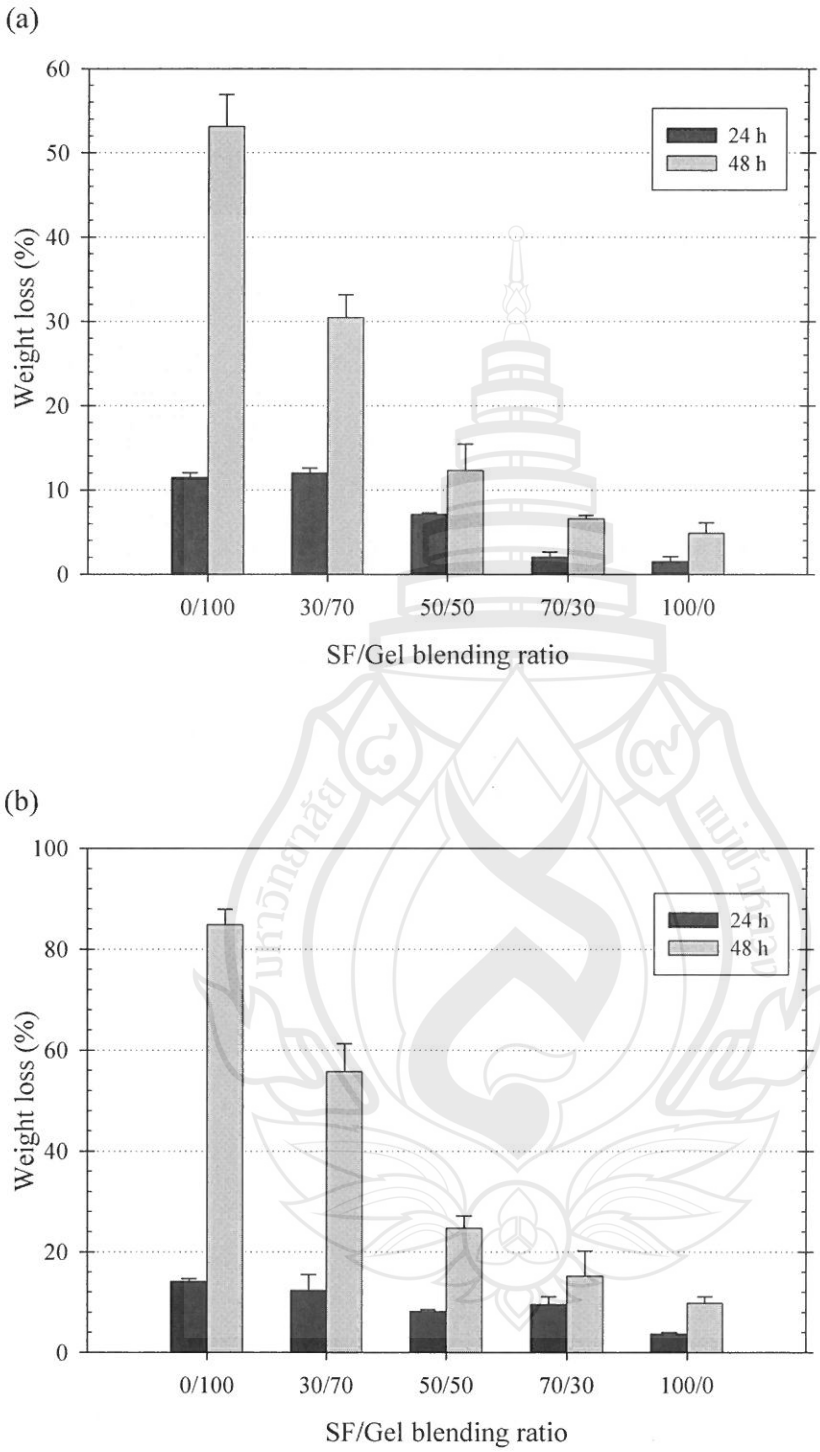


Figure 4.5 Weight loss of (a) the neat and (b) the GS-loaded SF/Gel blend scaffolds in phosphate buffer solution for 24 and 48 h.

4.6 Release of GS from GS-loaded SF/Gel blend scaffolds

The actual amounts of GS in the GS-loaded SF/Gel blend scaffolds were determined prior to investigating the release characteristics of GS from these samples and the results are shown in Table 4.4. The results showed that the actual amount of GS in the GS-loaded SF/Gel blend scaffold at the blending ratio of 0/100, 70/30, 50/50, 30/70, and 100/0 was ~95, ~92, ~98, ~94, and ~98% (based on the amounts of GS initially present in the GS-loaded SF/Gel blend scaffolds), respectively. These values were later used to calculate the cumulative amount of GS released from these the GS-loaded SF/Gel blend scaffold.

The release characteristics of GS from the GS-loaded SF/Gel blend scaffolds were investigated by the total immersion method over a period of 2880 min in the phosphate buffer solution. The cumulative release profiles of GS from the GS-loaded SF/Gel blend scaffolds were determined as the percentage corresponding to the weight of GS released divided by the actual weight of GS in the sample and the results are shown in Figure 4.6. In all cases, a gradual increase in the amount of GS released from these scaffolds was observed and more gradually increased afterwards. The cumulative amount of GS released seemed to plateau towards the end of the observational time period. The maximum amount of GS released from the GS-loaded SF/Gel blend scaffolds at the blending ratio of 0/100, 70/30, 50/50, 30/70, and 100/0 was ~80, ~73, ~64, ~61, and ~53%, respectively. The increasing amount of SF caused the cumulative amount of GS released from these scaffolds to decrease. These results might be explained by the weight loss results. Increasing the SF content decreased the weight loss of scaffolds.

Table 4.4 Actual amounts of GS incorporated in the GS-loaded SF/Gel blend scaffolds

GS-loaded SF/Gel blend scaffolds	Actual amount of GS based on the initial amount of GS loaded (%)
0/100	95.16 ± 5.33
30/70	92.12 ± 3.16
50/50	98.20 ± 3.16
70/30	94.15 ± 5.47
100/0	98.20 ± 3.82

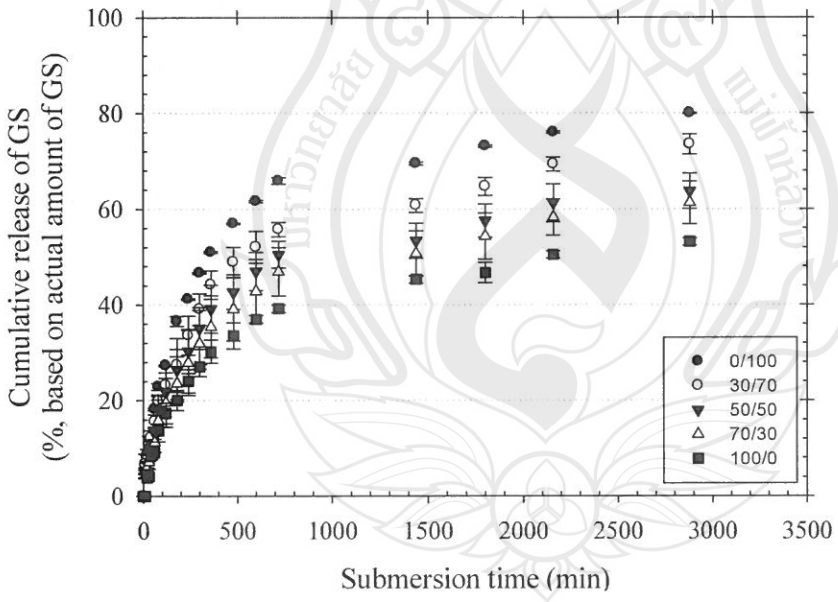


Figure 4.6 Cumulative release profile of GS from the GS-loaded SF/Gel blend scaffolds, reported as the percentage of the weight of GS released divided by the actual weight of GS in the samples, by total immersion method in phosphate buffer solution at the physiological temperature of 37 °C.

4.6 Antibacterial evaluation of GS-loaded SF/Gel blend scaffolds

Five candidate bacteria (i.e., *P. aeruginosa* as Gram-negative and *S. aureus*, *S. epidermidis*, *M. luteus*, and *B. cereus* as Gram-positive) were used to evaluate the antibacterial activity of the GS-loaded SF/Gel blend scaffolds. The percentage of inhibition or colony count provides a quantitative procedure for evaluation of the antibacterial activity of the GS-loaded SF/Gel scaffolds and the results are shown in Table 4.5. According to these results, the neat SF/Gel blend scaffolds (i.e., control) showed no activity against the growth of all bacteria. The GS-loaded SF/Gel blend scaffolds at blending ratio of 0/100 showed high activity against the growth of *S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus,* and *P. aeruginosa* with percent reduction of 99.94, 99.99, 100.00, 84.49, and 99.12, respectively. The GS-loaded SF/Gel blend scaffolds at blending ratio of 50/50 showed high activity against the growth of *S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus,* and *P. aeruginosa* with percent reduction of 99.99, 99.99, 99.99, 99.99, and 99.95, respectively. In addition, the GS-loaded SF/Gel blend scaffolds at blending ratio of 100/0 showed high activity against the growth of *S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus,* and *P. aeruginosa* with percent reduction of 99.99, 100.00, 99.88, 99.99, and 99.98, respectively. From these results, these scaffolds had high antibacterial activity against *S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus,* and *P. aeruginosa* indicating these scaffolds could be used as wound dressings.

Table 4.5 Antibacterial activity of the GS-loaded SF/Gel blend scaffolds (n = 3).

Culture	SF/Gel		CFU/mL (mean ± S.D.)	Percent reduction (%R)
S. aureus	100/0	Control	$6.8x10^7 \pm 0.9x10^7$	
		Treatment	$3.9x10^4 \pm 1.5x10^4$	99.94
	50/50	Control	$6.9x10^8 \pm 1.1x10^8$	
		Treatment	10.00 ± 17.0	99.99
	0/100	Control	$1.6x10^8 \pm 0.1x10^8$	
		Treatment	4.0 ± 6.0	99.99
S. epidermidis	100/0	Control	$5.40x10^8 \pm 0.3x10^8$	
		Treatment	$2.0x10^4 \pm 0.3x10^4$	99.99
	50/50	Control	$6.30 x10^8 \pm 0.2x10^8$	
		Treatment	3.0 ± 6.0	99.99
	0/100	Control	$5.1x10^8 \pm 0.4x10^8$	
		Treatment	0.0 ± 0.0	100.00
P. aeruginosa	100/0	Control	$5.8x10^9 \pm 5.0 x10^9$	
		Treatment	$5.0x10^7 \pm 0.4x10^7$	99.12
	50/50	Control	$4.0x10^9 \pm 3.5x10^9$	
		Treatment	$1.9x10^6 \pm 0.1x10^6$	99.95
	0/100	Control	$5.1x10^9 \pm 5.5x10^9$	
		Treatment	$3.5x10^2 \pm 0.7x10^2$	99.98
M. luteus	100/0	Control	$8.3x10^7 \pm 0.2x10^7$	
		Treatment	$9.5x10^4 \pm 0.2x10^4$	100.00
	50/50	Control	$8.5x10^7 \pm 0.5x10^7$	
		Treatment	17.0 ± 12.0	99.99
	0/100	Control	$9.3x10^7 \pm 0.4x10^7$	
		Treatment	0.0 ± 0.0	99.88
B. cereus	100/0	Control	$5.0x10^8 \pm 0.1x10^8$	
		Treatment	$6.5x10^7 \pm 2.3x10^7$	84.49
	50/50	Control	$3.9 x10^8 \pm 1.7x10^7$	
		Treatment	10.0 ± 17.0	99.99
	0/100	Control	$6.3x10^8 \pm 0.1x10^8$	
		Treatment	27.0 ± 46.0	99.99

4.7 Indirect cytotoxicity evaluation of GS-loaded SF/Gel blend scaffolds

To evaluate both the neat and the GS-loaded SF/Gel blend scaffolds could be used as wound dressings, indirect cytotoxicity evaluation was carried out on these scaffolds. The GS-loaded SF/Gel blend scaffolds were evaluated for their cytotoxicity. The viability of NHDF cells cultured with the extraction media from these samples in comparison with that of the cells cultured with the fresh culture medium is shown in Figure 4.7. The viability of the cells cultured with all the extraction media from the GS-loaded SF/Gel blend scaffolds at blending ratio of 0/100, 30/70, 50/50, 70/30, and 100/0 was ~103, ~117, ~96, ~88, and 59%, indicating that all the GS-loaded SF/Gel blend scaffolds were proven non-toxic to NHDF cells except the GS-loaded SF/Gel blend scaffolds at blending ratio of 100/0. Thus, these scaffolds might have potential use for wound dressings except the GS-loaded SF/Gel blend scaffolds at blending ratio of 100/0.

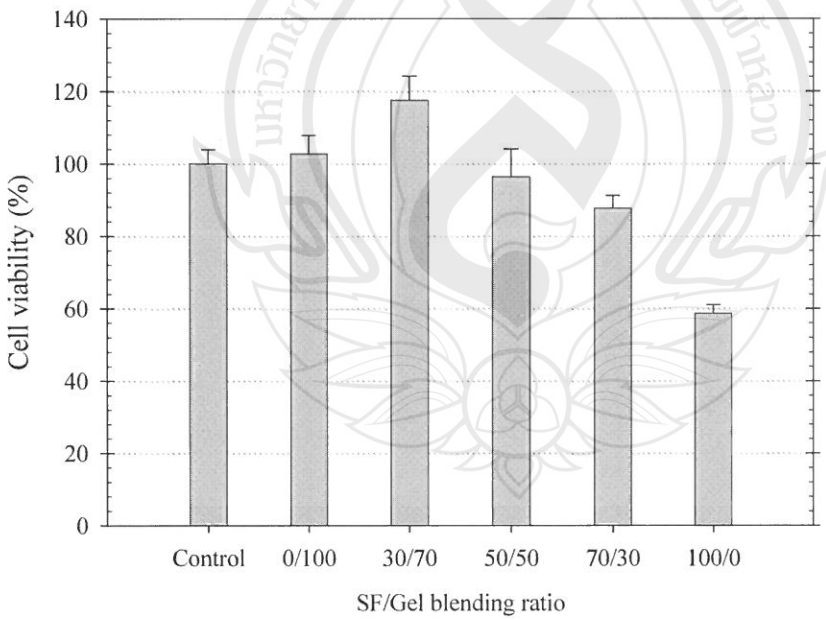


Figure 4.7 Indirect cytotoxicity evaluation of the GS-loaded SF/Gel scaffolds (n = 3).

CHAPTER 5
CONCLUSION

In this study, both the neat the GS-loaded SF/Gel blend scaffolds were successfully fabricated by freeze-drying method. GS was loaded into the neat SF/Gel blend scaffolds for use as wound dressings. The effect of SF/Gel blending ratio (i.e, 0/100, 30/70, 50/50, 70/30 and 100/0) in scaffolds on the morphology, water swelling, weight loss and release study was investigated. From SEM results, both the neat and the GS-loaded SF/Gel blend scaffolds had the interconnected porous structure. The pore size of the neat SF/Gel blend scaffolds ranged between 60 and 138 μm, while that of the GS-loaded SF/Gel blend scaffolds ranged between 77 and 100 μm. Increasing SF content decreased the compressive modulus of scaffolds. Moreover, the addition of GS into scaffolds caused the compressive modulus of scaffolds to decrease. The increase in the submersion time caused the more water molecule to diffuse into the scaffolds leading to increase both the water swelling and the weight loss. The addition of GS into scaffolds caused both the water swelling and the weight loss to increase. Because GS molecules which are good water soluble are highly diffuse into phosphate buffer solution. The cumulative amounts of GS released from the GS-loaded SF/Gel blend scaffolds decreased with an increase of SF content in scaffolds. All scaffolds showed high activity against the growth of *S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus,* and *P. aeruginosa*. Lastly, all the GS-SF/Gel blend scaffolds were proven non-toxic to NHDF cells except for the GS-loaded SF/Gel blend scaffolds at blending ratio of 100/0.

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BIOGRAPHY

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Presentation

1. N. Jaikaew, P. Supaphol, and **O. Suwantong**. (2013 April 23) Preparation and characterization of poly(L-lactic acid)-sericin hybrid scaffolds containing gentamicin sulfate for bone tissue engineering. Poster presentation at The 4th Research Symposium on Petrochemcial and Materials Technology and The 19th PPC Symposium on Petroleum, Petrochemicals and Polymers, Bangkok, Thailand.

2. P. Kudithalert, P. Supaphol, and **O. Suwantong**. (2013 April 23) Silk fibroin/gelatin blend scaffolds containing gentamicin sulfate for use as wound dressings. Poster presentation at The 4th Research Symposium on Petrochemcial and Materials Technology and The 19th PPC Symposium on Petroleum, Petrochemicals and Polymers, Bangkok, Thailand.
3. W. Kiatkontod and **O. Suwantong**. (2013 April 23) Preparation and characterization of alginate beads containing carbendazim for agricultural applications. Poster presentation at The 4th Research Symposium on Petrochemcial and Materials Technology and The 19th PPC Symposium on Petroleum, Petrochemicals and Polymers, Bangkok, Thailand.
4. K. Chuayboonsong, U. Ruktanonchai, and **O. Suwantong**. (2013 April 23) Controlled release of nanoemulsion for use as mosquito repellant products. Poster presentation at The 4th Research Symposium on Petrochemcial and Materials Technology and The 19th PPC Symposium on Petroleum, Petrochemicals and Polymers, Bangkok, Thailand.
5. N. Warakorn, P. Pankongadisak, P. Supaphol, and **O. Suwantong**. (2013 January 23-25) Resveratrol-loaded gelatin films and their potential for use as wound dressings. Poster presentation at The 7th Pure and Applied Chemistry International Conference (PACCON 2013), Chon Buri, Thailand.
6. P. Pankongadisak, U. Ruktanonchai, and **O. Suwantong**. (2013 January 23-25) Silver nanoparticles-loaded calcium alginate beads embedded in gelatin scaffolds and their release characteristics. Poster presentation at The 7th Pure and Applied Chemistry International Conference (PACCON 2013), Chon Buri, Thailand.
7. N. Jaikaew and **O. Suwantong**. (2012 November 29- December 1) Preparation and characterization of poly(L-lactic acid) scaffold by salt-leaching method. Poster presentation at 1st Mae Fah Luang University International Conference 2012 (MFUIC 2012), Chiang Rai, Thailand.

8. P. Pankongadisak, U. Ruktanonchai, and **O. Suwantong**. (2012 November 29- December 1) Preparation and characterization of silver nanoparticles-loaded calcium alginate beads embeded in gelatin scaffolds. Poster presentation at 1st Mae Fah Luang University International Conference 2012 (MFUIC 2012), Chiang Rai, Thailand.
9. W. Kiatkhontod and **O. Suwantong**. (2012 November 29- December 1) Preparation and characterization of carbendazim-loaded alginate beads. Poster presentation at 1st Mae Fah Luang University International Conference 2012 (MFUIC 2012), Chiang Rai, Thailand.
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11. **O. Suwantong**, S. Dechathai, and P. Supaphol. (2012 November 29-30) Release characteristics of electrospun poly(L-lactic acid) fiber mats containing acetone extracts of Garcinia mangostana. Poster presentation at The 24th Annual Meeting of the Thai Society for Biotechnology 2012 (TSB 2012), Ubon Ratchathani, Thailand
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