



ศูนย์วิจัยและส่งเสริมการศึกษา
มหาวิทยาลัยแม่ฟ้าหลวง

6.114800



รายงานวิจัยฉบับสมบูรณ์

ความเป็นไปได้ในการใช้วัสดุผสมพอลิแลคติก แอซิด-เซรีซินที่มียาเจนตา
มิซิน ซัลเฟต สำหรับประยุกต์ใช้เป็นวัสดุโครงร่างกระดูกเทียม

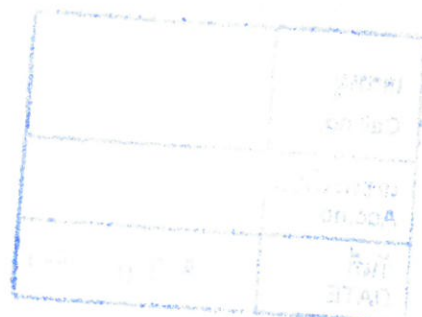
The potential use of poly(lactic acid)-sericin hybrid scaffolds
containing gentamicin sulphate for bone tissue engineering

โดย

ดร. อรรณพ สุวรรณทอง
ทญ. ดร. บุญทริกา ชื่นจิตกุลถาวร

งานวิจัยนี้ได้รับเงินอุดหนุนการวิจัยจากงบประมาณแผ่นดิน

ประจำปีงบประมาณ พ.ศ.2556



กิตติกรรมประกาศ (Acknowledement)

คณะวิจัยขอขอบคุณทุนอุดหนุนการวิจัย จากงบประมาณแผ่นดินประจำปีงบประมาณ 2556 สถาบันวิจัยวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย (วว.) สำหรับความอนุเคราะห์เชื้อแบคทีเรีย คือ *E. coli* TISTR 780 และ *S. aureus* TISTR 1466 ในการทดสอบฤทธิ์ต้านแบคทีเรียของวัสดุ และขอขอบคุณศูนย์เครื่องมือและเทคโนโลยี มหาวิทยาลัยแม่ฟ้าหลวง และมหาวิทยาลัยแม่ฟ้าหลวงที่ให้ความอนุเคราะห์ในการใช้สถานที่ และเครื่องมือในการทำวิจัยจนสำเร็จลุล่วงด้วยดี

อรรรรณ สุวรรณทอง

หัวหน้าโครงการวิจัย

วันที่ 30 เดือน กันยายน พ.ศ. 2556





บทสรุปผู้บริหาร

เงินอุดหนุนโครงการวิจัย จากงบประมาณแผ่นดิน

ประจำปีงบประมาณ พ.ศ. 2556

ตั้งแต่เดือน ตุลาคม พ.ศ. 2555 ถึง เดือน ตุลาคม พ.ศ. 2556

1. ชื่อโครงการ (ภาษาไทย) ความเป็นไปได้ในการใช้วัสดุผสมพอลิแลคติก แอซิด-เซรีซินที่มียาเจนตามิซิน ซัลเฟต สำหรับประยุกต์ใช้เป็นวัสดุโครงร่างกระดูกเทียม

ชื่อโครงการ (ภาษาอังกฤษ) The potential use of poly(lactic acid)-sericin hybrid scaffolds containing gentamicin sulphate for bone tissue engineering

2. ชื่อหัวหน้าโครงการ/ สำนักวิชา/ สัดส่วนการทำวิจัย

2.1 หัวหน้าโครงการ

ดร. อรรณพ สุวรรณทอง
สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง
สัดส่วนที่ทำการวิจัย 80%

2.2 ผู้ร่วมโครงการ

ทญ. ดร. บุญทริกา ชื่นจิตกุลถาวร
คณะทันตแพทยศาสตร์ มหาวิทยาลัยนเรศวร
สัดส่วนที่ทำการวิจัย 20%

3. ความสำคัญของปัญหาในการวิจัย

ในปัจจุบัน ประเทศไทยต้องนำเข้าวัสดุทางการแพทย์จากต่างประเทศ เพื่อใช้ตอบสนองความต้องการของประชาชนในประเทศ โดยต้องเสียค่าใช้จ่ายในการนำเข้าวัสดุทางการแพทย์ในราคาที่สูง ทำให้ไม่สามารถตอบสนองความต้องการของประชาชนที่มีรายได้ต่ำ ดังนั้นจึงมีความสนใจที่จะวิจัยพัฒนาวัสดุทางการแพทย์เพื่อทดแทนและลดการนำเข้าวัสดุทางการแพทย์จาก

ต่างประเทศ ซึ่งในงานวิจัยนี้ทำการขึ้นรูปวัสดุสำหรับใช้เป็นวัสดุโครงร่างกระดูกเทียม โดยเลือกใช้พอลิเมอร์ 2 ชนิด คือ poly(lactic acid) และ sericin สำหรับใช้ขึ้นรูปเป็นวัสดุโครงร่างกระดูกเทียม เนื่องจากพอลิเมอร์ทั้ง 2 ชนิดนี้ ได้มาจากธรรมชาติ จึงมีสมบัติที่ดี คือ ย่อยสลายได้เองตามธรรมชาติ เข้ากันได้กับเนื้อเยื่อ และไม่เป็นพิษต่อเซลล์เนื้อเยื่อ จากสมบัติดังที่กล่าวมาข้างต้น ทำให้ poly(lactic acid) เหมาะสำหรับใช้เป็นวัสดุทางการแพทย์ โดยเฉพาะใช้เป็นวัสดุโครงร่างกระดูกเทียม นอกจากนี้ sericin ที่นำมาใช้ในงานวิจัยนี้ ยังมีฤทธิ์ต้านอนุมูลอิสระ ฤทธิ์ต้านเชื้อแบคทีเรีย ดูดซึมและปล่อยความชื้นได้ดีอีกด้วย จึงทำให้ sericin เหมาะสำหรับใช้ในทางการแพทย์ อีกทั้งยังสามารถนำไปใช้ทางด้านความงาม เช่น เครื่องสำอาง แชมพู โลชั่น และยาทาเล็บ เนื่องจากมีสมบัติในการช่วยความยืดหยุ่นของผิวหนัง ต้านรอยเหี่ยวย่นของผิวหนัง และช่วยให้ความชุ่มชื้นแก่ผิวหนัง นอกจากนี้ sericin ยังเป็นวัสดุเหลือทิ้งจากโรงงานผลิตไหม ซึ่งอาจจะส่งผลเสียต่อสิ่งแวดล้อมโดยทำให้เกิดน้ำเสีย ดังนั้นเพื่อไม่ให้ sericin ถูกทิ้งไปโดยเปล่าประโยชน์ ในงานวิจัยนี้จึงได้มีการนำ sericin มาขึ้นรูปเป็นวัสดุผสม อีกทั้งยังได้มีการใส่ยา gentamicin sulphate ลงไปในวัสดุผสม poly(lactic acid)-sericin ซึ่งตัวยานี้มีฤทธิ์ต้านแบคทีเรีย โดยใช้รักษาโรคติดเชื้อของกระดูก (osteomyelitis) ที่เกิดจากการติดเชื้อที่กระดูกเองหรือการติดเชื้อแพร่มาจากที่อื่นๆ ของร่างกาย โดยเชื้อที่พบบ่อยที่สุดในคนทั่วไปคือ Staphylococcus aureus นอกจากนี้ในงานวิจัยยังได้ทำการศึกษาลักษณะการปลดปล่อยยา gentamicin sulphate จากวัสดุผสมที่ขึ้นรูปได้ และศึกษาความสามารถในการนำไปประยุกต์ใช้เป็นวัสดุโครงร่างกระดูกเทียม โดยทดสอบฤทธิ์ต้านแบคทีเรีย และความเป็นพิษของวัสดุผสมที่มียา gentamicin sulphate ก่อนที่จะนำไปประยุกต์ใช้จริงกับมนุษย์ ดังนั้นถ้าผลงานวิจัยนี้มีความสำเร็จดังที่ผู้วิจัยมุ่งหวังไว้ ก็จะส่งผลให้สามารถนำงานวิจัยไปต่อยอดเพื่อให้ได้วัสดุโครงร่างกระดูกเทียมที่เหมาะสมสำหรับรักษาผู้ป่วย มีราคาถูกลง และเป็นการช่วยเพิ่มทางเลือกให้มากขึ้น อีกทั้งยังสามารถลดการนำเข้าวัสดุทางการแพทย์จากต่างประเทศได้ นอกจากนี้อาจจะนำองค์ความรู้ที่เกิดขึ้น ไปพัฒนาต่อยอดเป็นวัสดุโครงร่างกระดูกเทียมจากวัสดุชนิดอื่นๆ หรืออาจจะต่อยอดเป็นวัสดุทางการแพทย์อื่นๆ เช่น วัสดุปิดแผล เป็นต้น โดยอาจมีการผสมผสานเทคโนโลยีและภูมิปัญญาท้องถิ่น เช่น ใช้สมุนไพรไทยที่พบได้ในท้องถิ่นเป็นตัวยาสารสำหรับศึกษาฤทธิ์ต้านต่างๆ ซึ่งอาจก่อให้เกิดทางเลือกใหม่ๆ ขึ้นมาได้อย่างไม่มีที่สิ้นสุด

4. วัตถุประสงค์ของโครงการวิจัย

- 4.1 เพื่อขึ้นรูปวัสดุผสม poly(lactic acid)-sericin
- 4.2 เพื่อศึกษาลักษณะการปลดปล่อยยา gentamicin sulphate จากวัสดุผสม poly(lactic acid)-sericin
- 4.3 เพื่อศึกษาฤทธิ์ต้านเชื้อแบคทีเรีย และความเป็นพิษของวัสดุผสม poly(lactic acid)-sericin ที่มียา gentamicin sulphate

5. ขอบเขตของโครงการวิจัย

- 5.1 ศึกษาอัตราส่วนผสม และตัวแปรต่างๆที่ใช้ในการขึ้นรูปวัสดุ poly(lactic acid) ด้วยเทคนิค salt leaching
- 5.2 ทำการสกัดแยก sericin จาก silk protein
- 5.3 ขึ้นรูปวัสดุผสม poly(lactic acid)-sericin ที่มียา gentamicin sulphate ด้วยเทคนิค dipping และ freeze drying
- 5.4 ศึกษาลักษณะสัณฐานวิทยา ลักษณะรูพรุน ของวัสดุผสมที่ขึ้นรูปได้ ด้วย Scanning Electron Microscope (SEM)
- 5.5 ศึกษาปริมาณการกักเก็บน้ำ (Water retention) ของวัสดุผสมหลังจากแช่ในสารละลายบัฟเฟอร์ (Phosphate buffer solution, PBS)
- 5.6 ศึกษาสมบัติเชิงกล (Mechanical properties) ของวัสดุผสม poly(lactic acid)-sericin ที่มียา gentamicin sulphate
- 5.7 ศึกษาลักษณะการปลดปล่อยยา gentamicin sulphate (Release characteristics) จากวัสดุผสม หลังจากแช่ในสารละลายบัฟเฟอร์ (Phosphate buffer solution, PBS) ที่เวลาต่างๆ
- 5.8 ศึกษาฤทธิ์ต้านแบคทีเรียของวัสดุผสมที่มียา gentamicin sulphate
- 5.9 ศึกษาความพิษของวัสดุผสมที่มียา gentamicin sulphate ต่อเซลล์กระดูก (MC3T3-E1) ด้วยวิธี indirect cytotoxicity

6. ผลผลิตจากการวิจัย

วัสดุผสม poly(lactic acid)-sericin ที่ขึ้นรูปได้นี้ เป็นวัสดุนำส่งยา gentamicin sulphate เพื่อศึกษาลักษณะการปลดปล่อยยาจากวัสดุผสม ฤทธิ์ต้านเชื้อแบคทีเรีย และความเป็นพิษของวัสดุผสม ซึ่งวัสดุผสมที่ได้จากงานวิจัยนี้ สามารถนำไปใช้เป็นวัสดุโครงร่างเนื้อเยื่อเทียม โดยเฉพาะอย่างยิ่ง วัสดุโครงร่างกระดูกเทียม เนื่องจากยาปฏิชีวนะ gentamicin sulphate ที่ใช้ในวัสดุผสมนี้มีฤทธิ์ต้านเชื้อแบคทีเรีย ซึ่งสามารถช่วยรักษาโรคติดเชื้อของกระดูก (Osteomyelitis) ได้ จากการทดลองพบว่า วัสดุผสมที่ขึ้นรูปได้สามารถเป็นวัสดุนำส่งยา gentamicin sulphate ได้ และเมื่อทำการทดสอบฤทธิ์ต้านเชื้อแบคทีเรียของวัสดุผสมนี้ พบว่าวัสดุผสมมีความสามารถในการยับยั้งเชื้อแบคทีเรียได้ดี นอกจากนี้ เมื่อทำการทดสอบความเป็นพิษของวัสดุผสมนี้ พบว่า วัสดุผสมไม่มีความเป็นพิษต่อเซลล์กระดูก ดังนั้นวัสดุผสม poly(lactic acid)-sericin ที่มียา gentamicin sulphate เหมาะสมสำหรับนำไปประยุกต์ใช้เป็นวัสดุโครงร่างกระดูกเทียม เพื่อทดแทนวัสดุทางการแพทย์ที่นำเข้ามาจากต่างประเทศบางชนิดได้

7. ประโยชน์ที่ได้รับจากโครงการวิจัย ผลกระทบทางวิชาการหรือเทคโนโลยี

จากงานวิจัยนี้ ได้องค์ความรู้ใหม่ ซึ่งสามารถนำความรู้ที่ได้ไปต่อยอดโดยร่วมมือกับภาครัฐและเอกชนเพื่อผลักดันผลงานวิจัยเข้าสู่กระบวนการเชิงพาณิชย์ โดยผลิตเป็นวัสดุโครงร่างเนื้อเยื่อเทียม เช่น กระดูกเทียม เพื่อรักษาการติดเชื้อของกระดูกที่เกิดจากการแตกหักของกระดูก หรือจากการติดเชื้อของแบคทีเรีย ทั้งนี้เพื่อลดต้นทุนการผลิต และช่วยส่งเสริมคุณภาพชีวิตของผู้ป่วยให้ดีขึ้น



Abstract

Polymeric scaffold has been widely used as medical devices and tissue-engineering scaffolds. It has many advantages, for example, biodegradable, biocompatible, high surface-to-volume ratio, high porosity with very small pore size, and proper mechanical properties. Poly(L-lactic acid) (PLLA) is thermoplastic polyester derived from renewable resources, such as corn and starch. It has many properties such as biocompatible, biodegradable, and non-toxic to human body. Therefore, it has been used in tissue engineering. Sericin is a natural polymer derived from silk cocoon with the good advantages of antioxidant activity and moisture retention capacity. In this research work, the PLLA-sericin hybrid scaffolds containing gentamicin sulphate (GS) were fabricated for use in bone tissue engineering application. Since GS is an aminoglycoside antibiotic that is useful in skin/soft tissue, bone and severe respiratory infections. PLLA scaffolds were prepared by particulate leaching using sodium chloride (NaCl) with size of 300-425 μm as a particulate leaching. PLLA:NaCl ratio was varied as 1:8, 1:10, 1:12, and 1:15 at a fixed concentration of 20% w/v PLLA. The GS-loaded PLLA-sericin hybrid scaffolds were obtained by immersion of the PLLA scaffolds in sericin solution containing GS and subsequently freeze drying. The morphology and pore size of these scaffolds were characterized by Scanning Electron Microscopy (SEM). The water retention and weight loss behaviors and the compressive strength of these scaffolds were investigated. The release characteristics of GS from these scaffolds were carried out in phosphate buffer solution (PBS). Lastly, the antibacterial activity and the indirect cytotoxicity of these scaffolds were evaluated.

Key words: Poly(L-lactic acid), Scaffolds, Particulate Leaching, Sericin, Gentamicin Sulphate

บทคัดย่อ

วัสดุโครงร่างพอลิเมอร์นิยมใช้เป็นวัสดุทางการแพทย์ และวัสดุโครงร่างเนื้อเยื่อเทียม เนื่องจากมีข้อดีหลายประการ ยกตัวอย่างเช่น ย่อยสลายได้เองตามธรรมชาติ เข้ากันได้กับเนื้อเยื่อ มีอัตราส่วนพื้นที่ผิวต่อปริมาตรสูง มีความเป็นรูพรุนสูง และสมบัติเชิงกลเหมาะสม พอลิแลคติก แอซิด เป็นพอลิเอสเทอร์ที่ได้จากแหล่งทรัพยากรที่นำกลับมาใช้ใหม่ได้ เช่น ข้าวโพด และแป้ง พอลิแลคติก แอซิดมีสมบัติหลายประการ เช่น สามารถเข้ากันได้กับเนื้อเยื่อ ย่อยสลายได้เองตามธรรมชาติ และไม่เป็นพิษต่อร่างกายมนุษย์ ดังนั้นพอลิแลคติก แอซิดจึงถูกนำมาใช้ในวิศวกรรมเนื้อเยื่อ ขณะที่เซรีซินเป็นพอลิเมอร์ธรรมชาติได้มาจากรังไหม ซึ่งมีข้อดีหลายประการ คือ ต้านอนุมูลอิสระ และสามารถกักเก็บความชื้นได้ดี ในงานวิจัยนี้ ได้ทำการขึ้นรูปวัสดุผสมพอลิแลคติก แอซิด-เซรีซินที่มียาเจนตามิซินซัลเฟต สำหรับประยุกต์ใช้เป็นวัสดุโครงร่างกระดูกเทียม เนื่องจากยาเจนตามิซินซัลเฟต เป็นยาปฏิชีวนะที่สามารถยับยั้งเชื้อแบคทีเรียที่เกิดจากการติดเชื้อที่พบได้ในผิวหนัง หรือกระดูก พอลิแลคติก แอซิดถูกขึ้นรูปด้วยเทคนิค salt leaching โดยใช้โซเดียมคลอไรด์ที่มีขนาดประมาณ 300 ถึง 425 ไมโครเมตร ในอัตราส่วนของพอลิแลคติก แอซิดต่อโซเดียมคลอไรด์ เท่ากับ 1:8, 1:10, 1:12 และ 1:15 ขณะที่วัสดุผสมพอลิแลคติก แอซิด-เซรีซินที่มียาเจนตามิซินซัลเฟต ถูกขึ้นรูปโดยการแช่วัสดุพอลิแลคติก แอซิดลงในสารละลายเซรีซินที่มียาเจนตามิซินซัลเฟต จากนั้นนำไปขึ้นรูปด้วยกระบวนการ freeze drying ในงานวิจัยนี้ ได้ทำการศึกษาลักษณะพื้นฐานวิทยา และขนาดของรูพรุนของวัสดุผสมด้วยกล้องจุลทรรศน์แบบส่องกราด ศึกษาปริมาณการกักเก็บน้ำ การสูญเสียน้ำหนัก และความทนแรงอัดของวัสดุผสมที่ขึ้นรูปได้ ศึกษาลักษณะการปลดปล่อยยาเจนตามิซินซัลเฟตจากวัสดุผสมในสารละลายฟอสเฟตบัฟเฟอร์ ศึกษาฤทธิ์การยับยั้งเชื้อแบคทีเรีย และความเป็นพิษของวัสดุผสมที่ขึ้นรูปได้

คำสำคัญ: พอลิแลคติก แอซิด วัสดุโครงร่าง การแช่แข็งด้วยอนุภาค เซรีซิน เจนตามิซินซัลเฟต

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CHAPTER 1

INTRODUCTION

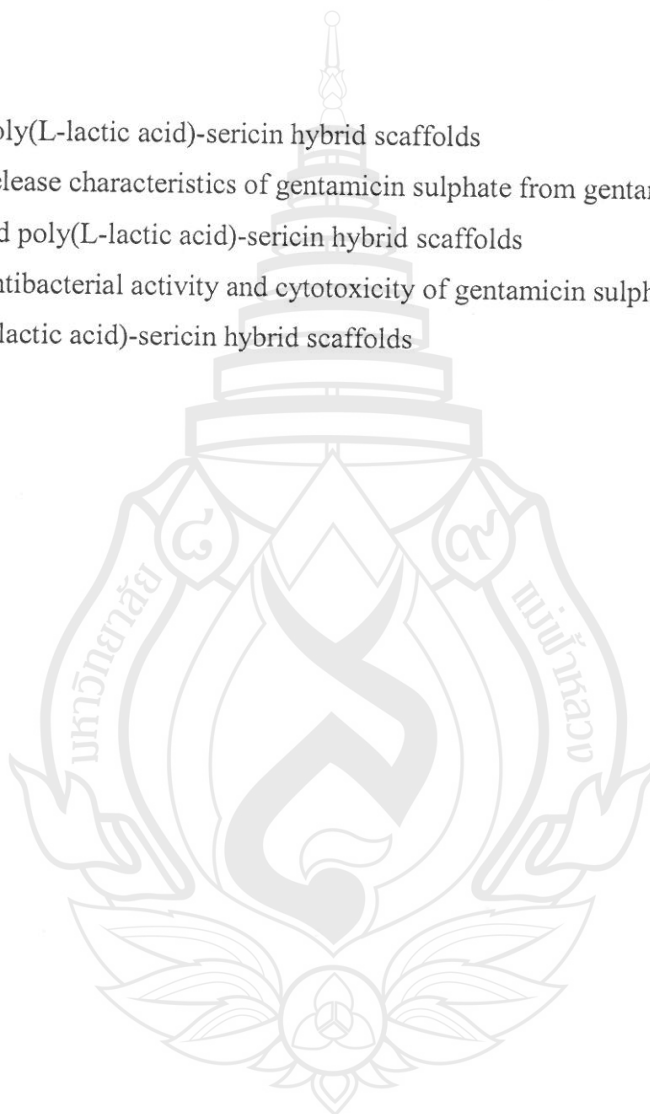
1.1 Statement and significance of the problem

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, poly(lactic acid) and sericin are used to fabricate the bone tissue engineering scaffolds. Since these polymers have several properties such as biodegradable, biocompatible, and non-toxic to human cells. From these properties, poly(lactic acid) is suitable for use as biomedical materials, especially bone tissue engineering scaffolds. Moreover, sericin used in this research has several properties such as good water absorption, antioxidant and antibacterial properties. Thus it can be used in biomedical applications. It also can be used in cosmetic industries such as shampoo, lotion, cosmetics for skin, hair, and nails since it helps to enhance the elasticity of skin and has antiwrinkle and antiaging properties. Moreover, it is an unutilized byproduct of the textiles industry and is produced during the processing of raw silk cocoons. The discarded sericin in wastewater after silk processing leads to environmental contamination. Thus in this research, we use sericin as a hybrid material and also add gentamicin sulphate into poly(lactic acid)-sericin hybrid scaffolds. Gentamicin sulphate (GS) is antibiotic drug which has antibacterial activities. It is used to treat osteomyelitis that caused by bacterial infection of the bone medullar cavity, cortex and/or periosteum or caused by post operative sepsis following orthopedic surgery. Osteomyelitis can occur in bone and its marrow after knee or hip replacement surgery mainly by pyrogenic microorganism, *Staphylococcus aureus*. In this research, the release characteristics of GS from poly(lactic acid)-sericin hybrid scaffolds were investigated. Moreover, the antibacterial and cytotoxicity tests of these materials were investigated for use in bone tissue engineering applications. If this research is successfully as researcher expected, it can lead to further research in order to obtain the low cost and suitable bone tissue

engineering scaffolds 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 poly(L-lactic acid)-sericin hybrid scaffolds
2. To study the release characteristics of gentamicin sulphate from gentamicin sulphate-loaded poly(L-lactic acid)-sericin hybrid scaffolds
3. To study the antibacterial activity and cytotoxicity of gentamicin sulphate-loaded poly(L-lactic acid)-sericin hybrid scaffolds



CHAPTER 2

LITERATURE REVIEWS

Bone infection is a significant problem that comes from the healing of bone fractures. Osteomyelitis is a bone disease caused by bacterial infection of bone mainly by pyrogenic microorganism, *Staphylococcus aureus*. One of its most common causes is post-operative sepsis following orthopedic surgery. Normally, preventative treatments against post-operative infection have been treated with antibiotic therapy for 4-6 weeks (Coombos and Fitzgerald, 1989; Sampath et al., 1992; Schlossberg, 1984). However, it is difficult to achieve a sufficient concentration of antibiotics at the infection site by systematic administration of the drug. Thus, controlled release of antibiotics at the infection site is new development being employed to treat chronic infections such as osteomyelitis (Graham and McNeill, 1984; Kopecek, 1984). Controlled delivery 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 improvement 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.

Tissue engineering is the science of design and fabrication of new tissues for functional restoration of impaired organs and replacement of lost parts due to cancer, disease and trauma. Among many tissues in the body, bone has the highest potential for regeneration and therefore is a prototype paradigm for the enunciation of principles of tissue engineering in general. The accumulating knowledge in tissue engineering will lead to the design of bone with predetermined shapes for orthopaedic surgery applications. Bone is a dynamic and highly vascularized tissue that continues to remodel throughout the lifetime of an individual. It plays an integral role in locomotion, ensures the skeleton has adequate load-bearing capacity, and acts as a protective casing for the delicate internal organs of the body (Langer and Vacanti, 1993; Nerem, 1995).

Scaffold is a material with highly porous structure that can control release of growth factors over the period of tissue regeneration. Tissue engineering has emerged as one such promising alternative approach to treat patients suffering from the problem of medical device which is not able to replace all the function of damage or loss of organ or tissue. Tissue engineering involves the expansion of cells in temporary three-dimensional scaffolds to form the new organ or tissue. With this approach, porous three-dimensional temporary scaffolds play an important role in manipulating cell functions. Isolated and expanded cell adhere to porous three-dimensional temporary scaffolds, proliferate, and secrete their own extracellular matrices (ECM). Therefore, in addition to permit cell adhesion, promoting cell growth, and allowing retention of differentiated cell functions, the scaffold should be biocompatible, biodegradable, highly porous with a large surface to volume ratio, mechanically strong and capable of being formed into design shapes (Chen et al., 2001). In addition to being biocompatible both in bulk and degraded forms, these scaffolds should possess appropriate mechanical properties to provide the correct stress environment for the tissues. Also, the scaffolds should be porous and permeable to permit the ingress of cells and nutrients, and should exhibit the appropriate surface structure and chemistry for cell attachment. Scaffold is made from several different materials, depending on the type of cell doctor need to grow. The most popular material is a biodegradable polymer. These polymers gradually dissolved in the body as new tissue develops and no longer need support (Maurus and Kaeding, 2004).

Biomaterials play a critical role in the tissue engineering by acting as synthetic frameworks referred as scaffolds, matrices, or constructs. In recent years, there has been increasing importance on materials that could be used in biomedical areas. Biomaterials used in biomedical applications target to develop artificial materials that can be used to renovate or restore function of diseased or traumatized tissues in the human body and thus improve the quality of life. The biomaterials selection based on availability, design attempts were primarily focused on either achieving structural/mechanical performance or on rendering biomaterials inert and thus unrecognizable as foreign bodies by the immune system. Biomaterials are used as implants in the form of sutures, bone plates, joint replacements, ligaments, vascular grafts, heart, intraocular lenses, dental implants, and medical devices like pacemakers

and biosensors (Hutmacher, 2001). The three-dimensional porous scaffolds with higher porosities having homogeneous interconnected pore network are highly useful for tissue engineering.

The selection of the most appropriate material to produce a scaffold to be used in bone tissue engineering applications is very important step towards the construction of a tissue engineering product. Basic requirements for designing polymer scaffolds are biocompatible and biodegradable. For biocompatibility, the polymer scaffolds should not invoke an adverse inflammatory or immune response. The factors that determine its biocompatibility such as the chemical structure and morphology can be affected by polymer synthesis and scaffold processing. In addition toxic residual chemicals involved in these processes such as monomers stabilizer, initiator, cross-linking agents and organic solvent can be effects on the surrounding tissue as well. For biodegradable property, the polymer should contain the hydrolysable linkages on the polymer backbone and their degraded products can be eliminated from the human body (Rosso et al., 2005).

Poly(L-lactic acid) or PLLA is a family of linear aliphatic polyester that has been extensively studied to fabricate scaffolds because of its advantages of availability ease of processing, adjustable degradation, low inflammatory response. In addition, the mechanical property and biodegradability of PLLA can be readily regulated by controlling its molecular weight through its synthesis conditions. For the biodegradation property, when PLLA is implanted in the body, hydrolysis of polymer backbone reduces its molecular weight and its degrade product of lactic acid was eliminated from the body by incorporation into the tricarboxylic acid cycle (Fambri et al., 1997; Renouf-Glauser et al., 2005). Thus, PLLA has potential for use in biomedical applications, especially bone tissue engineering (Giurea et al., 2003; Hsu et al., 2011; Li et al., 2012; Long et al., 2012).

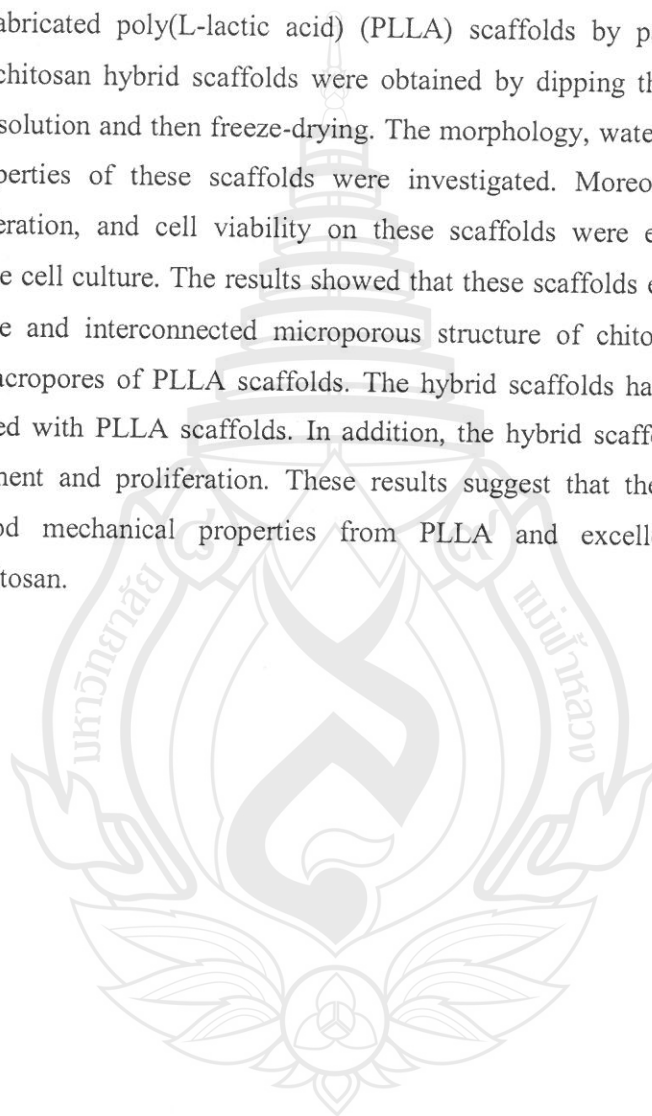
Sericin is a macromolecular protein. Its molecular weight ranges from about 10 to over 300 kDa. It can crosslink, copolymerize and blend with other polymers to produce biodegradable materials because of its highly hydrophilic with strong polar side chain such as hydroxyl, carboxyl and amino groups. In addition, is has several properties such as good water absorption, antioxidant, antibacterial properties, and also good biocompatibility and biodegradability. It also can be used in cosmetic

industries such as shampoo, lotion, cosmetics for skin, hair, and nails since it helps to enhance the elasticity of skin and has antiwrinkle and antiaging properties (Chang-Kee et al., 2002; Kato et al., 1998; Ogawa and Yamada, 1999; Padamwar and Pawar, 2004; Voegeli et al., 1993; Yamada et al., 1998; Zhang, 2002).

Gentamicin sulphate (GS) is an aminoglycoside antibiotic with a wide antibacterial spectrum, has been incorporated into scaffolds or bone repairing materials to prevent infections. The incorporation of antibiotics in scaffolds for the treatment and prevention of infection in orthopaedics such as osteomyelitis has become common clinical practice. GS has been the most widely used antibiotic in controlled release devices, due to its broad-spectrum antimicrobial activity, excellent solubility and stability at elevated temperatures (Frutos et al., 2002).

From these literature reviews, Changez et al. (2003) prepared poly(acrylic acid)-gelatin hydrogel containing GS for treatment of osteomyelitis. In later year, Changez et al. (2004) prepared interpenetrating network hydrogels (IPNs) based on poly(acrylic acid) and gelatin containing GS for evaluation of the release of GS. They also studied in vitro and vivo degradation of these hydrogels. From the results, these hydrogels have a potential for the treatment of localized infection like osteomyelitis. Moreover, Sato et al. (2004) prepared PLLA-collagen hybrid scaffolds by salt leaching method for use as cartilage tissue engineering. They implanted these scaffolds in the dorsa of athymic nude mice to tissue engineer articular cartilage in vivo. The results showed that the PLLA-collagen scaffolds retained the advantages of both PLLA and collagen, and facilitated the formation of cartilaginous tissue, suggesting that it would be suitable scaffolds for cartilage tissue engineering. In 2007, Prabakaran et al. fabricated poly(L-lactic acid)-chitosan hybrid scaffolds to be used as tissue engineering scaffolds and drug release carriers. In this research work, an anti-inflammatory drug, ketoprofen, was used as a model drug to study the release characteristics of ketoprofen from these scaffolds. The results showed that the drug release rate can be controlled by the chitosan content and cross-link densities, suggesting the potential of these hybrid scaffolds as drug delivery systems and tissue engineering scaffolds. The porous scaffolds were prepared using biodegradable polylactide/chitosan blends by a combinational technique involving solvent-extracting, liquid-solid separation, and freeze-drying methods (Wan et al., 2007). The

obtained scaffolds showed well distributed and interconnected porous structures with controllable porosities varying from around 50-85%. Moreover, these scaffolds maintained good compressive mechanical properties and by well-blending polylactide with chitosan component, improved toughness on the scaffolds were also obtained. Jiao et al. (2007) fabricated poly(L-lactic acid) (PLLA) scaffolds by particulate leaching and PLLA-chitosan hybrid scaffolds were obtained by dipping the PLLA scaffolds in chitosan solution and then freeze-drying. The morphology, water uptake, and mechanical properties of these scaffolds were investigated. Moreover, cell adhesion, cell proliferation, and cell viability on these scaffolds were evaluated through osteoblast-like cell culture. The results showed that these scaffolds exhibited macroporous structure and interconnected microporous structure of chitosan was formed within the macropores of PLLA scaffolds. The hybrid scaffolds had higher water uptake compared with PLLA scaffolds. In addition, the hybrid scaffolds had excellent cell attachment and proliferation. These results suggest that the hybrid scaffolds obtain good mechanical properties from PLLA and excellent cell compatibility from chitosan.



CHAPTER 3

METHODOLOGY

3.1 Materials

Poly(L-lactic acid) (PLLA; intrinsic viscosity = 1.28 dL.g^{-1}) was obtained from NatureWorks LLC (USA). Dichloromethane was purchased from Labscan (Thailand). Sodium chloride (NaCl) was purchased from Ajax Chemicals (Australia). Glutaraldehyde solution was purchased from Fluka Analytical (Switzerland). Gentamicin sulfate (GS) powder was obtained from Shijiazhuang Pharm-chem Technology (China).

3.2 Preparation of sericin solution

Silk cocoons were cut into small pieces, boiled with Na_2CO_3 for 45 min twice for degumming and then washed with deionized water in several time to remove sericin out. Sericin solution was dialyzed with deionized water for 5 days by changing deionized water every 3 h to remove solvent. Sericin solution was centrifuged at 2,000 rpm for 20 min at 25°C . Then, sericin solution was lyophilized by using freeze-drying for 20 h. Fourier transform infrared spectroscopy (FTIR) analysis of the obtained sericin was carried out using an FTIR spectrometer (Spectrum GX, Perkin-Elmer) in the spectral region of $4000 - 400 \text{ cm}^{-1}$ with 4 cm^{-1} resolution.

3.3 Preparation of neat and GS-loaded PLLA-sericin hybrid scaffolds

Both of the neat and the GS-loaded PLLA-sericin hybrid scaffolds were fabricated by particulate leaching and dipping methods. NaCl particles sieved in the range of $300\text{-}425 \text{ }\mu\text{m}$ were used as leaching particles. First, PLLA pellets were dissolved in dichloromethane at a fixed concentration of $20 \text{ \%w/v}$ PLLA. The NaCl particles were then added into the PLLA solution at different weight ratios of PLLA:NaCl particles (i.e., 1:8, 1:10, 1:12, and 1:15). The pasty suspensions were filled in glass mold and Petri dishes which are two different shapes and dimensions of the molding samples. The molding samples were placed in a fume hood overnight to

evaporate solvent. NaCl particles were then leached out by immersing the molding in deionized (DI) water for 48 h, with DI water being replaced every 4 h, then freeze-dried to obtain the neat PLLA scaffolds.

The GS-loaded PLLA-sericin hybrid scaffolds were prepared by immersing the neat PLLA scaffolds in 1%w/v sericin solution containing 5 mg·mL⁻¹ of GS and 0.02% v/v glutaraldehyde under vacuum system. Then the GS-loaded PLLA-sericin hybrid scaffolds were obtained by freeze-drying method.

3.4 Morphological observation and pore size measurement

The morphological appearance of both the neat and the GS-loaded PLLA-sericin hybrid scaffolds was investigated by LEO 1450 VP Scanning Electron Microscope (SEM). Prior to observation, each sample was coated with thin layer of gold using a Polaron SC-7620 sputtering device. Pore sizes of these scaffolds were measured directly from SEM images using SemAphore 4.0 software. These values were averaged to obtain the pore sizes of particular pore. More than 30 pores of each sample were measured.

3.5 Water retention and weight loss

The water retention and the weight loss behaviors of the neat and the GS-loaded PLLA-sericin hybrid scaffolds were measured in a phosphate buffer solution at the physiological temperature of 37 °C for 24 and 48 h. The measurements of each sample were calculated according to the following equations:

$$\text{Water retention (\%)} = \frac{M - M_d}{M_d} \times 100, \quad (1)$$

$$\text{And Weight loss (\%)} = \frac{M_i - M_d}{M_i} \times 100, \quad (2)$$

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.

3.6 Mechanical test

The compressive modulus of both the neat and the GS-loaded PLLA-sericin hybrid 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 PLLA-sericin hybrid scaffolds were compressed at the crosshead speed of 1 mm·min⁻¹ until the samples were around 70% deformed from their original height of ~8 mm. The obtained data were modified by connecting with computer for control apparatus and analyzing of the results.

3.7 Release of GS from GS-loaded PLLA-sericin hybrid scaffolds

3.7.1 Actual GS content

The actual amount of GS in the GS-loaded PLLA-sericin hybrid 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 PLLA-sericin hybrid scaffolds was then back-calculated from the resulting data against a predetermined calibration curve.

3.7.2 GS release assay

The release characteristics of GS from the GS-loaded PLLA-sericin hybrid 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 PLLA-sericin hybrid scaffolds.

3.8 Antibacterial evaluation of GS-loaded PLLA-sericin hybrid 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 investigate the antibacterial activity of the GS-loaded PLLA-sericin hybrid scaffolds. The pathogenic bacteria, *Escherichia coli* TISTR 780 and *Staphylococcus aureus* TISTR 1466, 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 °C for 24 h. For control, the neat PLLA 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:

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

where A is the number of bacteria recovered from the incubated treated test sample (GS-loaded PLLA-sericin hybrid scaffolds) after 37 °C for 24 h and B is the number of bacteria recovered from the incubated untreated test sample (neat PLLA scaffolds) after 37 °C for 24 h.

3.9 Indirect cytotoxicity evaluation of neat and GS-loaded PLLA-sericin hybrid scaffolds

The indirect cytotoxicity evaluation of the GS-loaded PLLA-sericin hybrid scaffolds was conducted in 24-well tissue-culture polystyrene plate (TCPS; Corning Costar®, USA) using MC3T3-E1 (mouse osteoblast cell line). The cells were cultured in Modified Eagle's medium (MEM; GIBCO, USA), containing 10% fetal bovine serum (FBS; GIBCO, USA), 2 mM L-glutamine, 100 units/mL penicillin, 100 $\mu\text{g}\cdot\text{mL}^{-1}$ streptomycin, and 5 $\mu\text{g}\cdot\text{mL}^{-1}$ amphotericin B (GIBCO, USA) at 37°C in a humidified atmosphere of 95% air and 5% CO₂. The samples cut from the GS-loaded PLLA-sericin hybrid scaffolds were first sterilized by UV radiation for ~1 h and then immersed in serum-free medium (SFM; containing MEM, 1% L-glutamine and 1% antibiotic and antimycotic formulation) for 24 h in incubation to produce extraction media. MC3T3-E1 cells were separately cultured in wells of TCPS at 50,000 cells/well in serum-containing MEM 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 300 μL /well of MTT solution at 5 $\text{mg}\cdot\text{mL}^{-1}$. The plate was incubated further for 4 h at 37 °C. The solution was then aspirated and 300 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 xMark™ Microplate Absorbance Spectrophotometer (Bio-Rad, USA).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Preparation of sericin

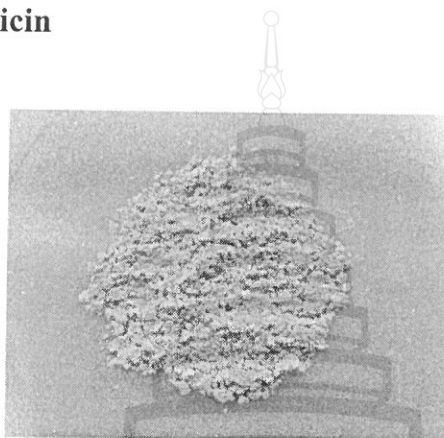


Figure 4.1 Sericin.

The sericin samples were characterized by using FTIR. The results showed the absorption between 1600 and 1700 cm^{-1} showing amide I absorption which comes from the C=O stretching vibration and is the most useful peak for determining secondary structure of protein. The peak at 1542.41 cm^{-1} corresponds to amide II which shows the presence of a random coil structure. Signature peak for sericin at 1395.08 cm^{-1} was also observed. Moreover, the absence of a shoulder peak at 1260 cm^{-1} due to aggregation of sericin molecules, not to β -sheet conformation (Teramoto and Miyazawa, 2005).

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 $\text{CaCl}_2\text{:EtOH:H}_2\text{O}$ 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).

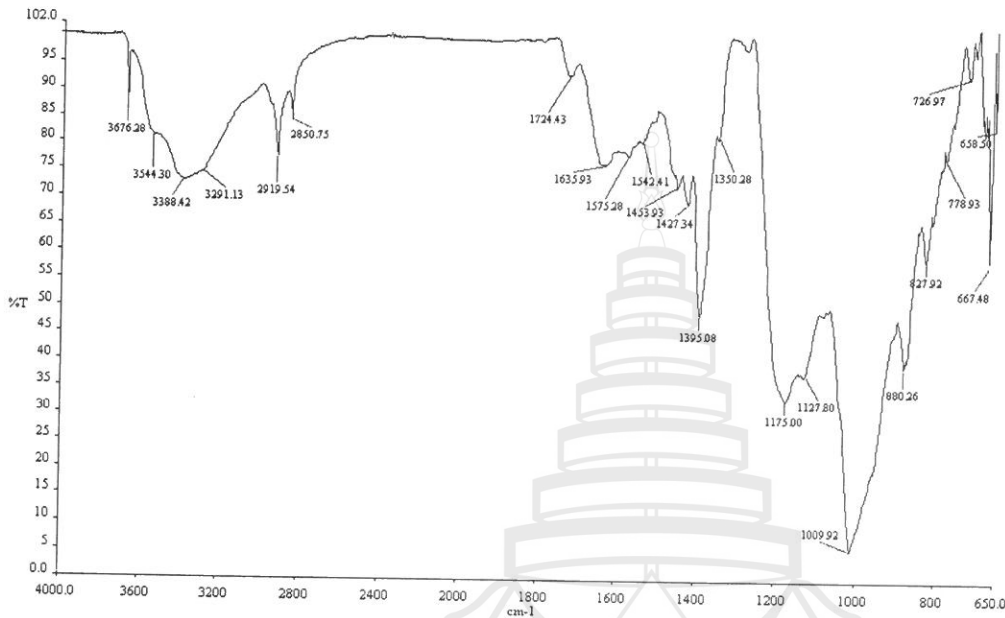
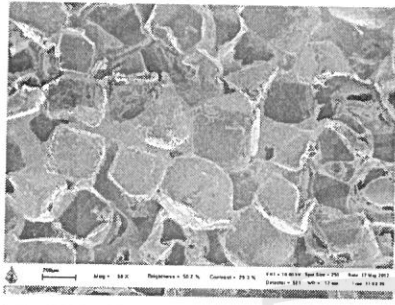
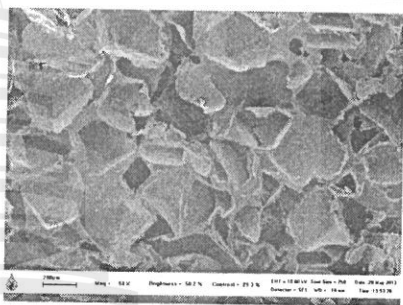
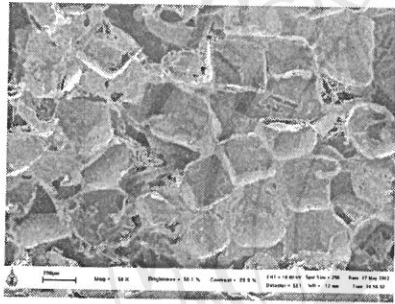
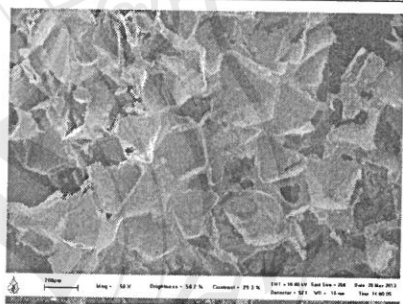
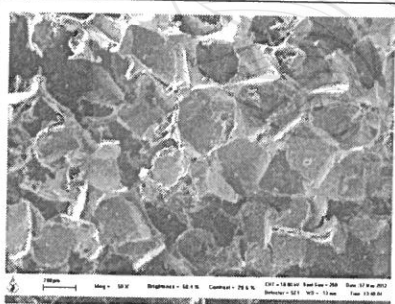
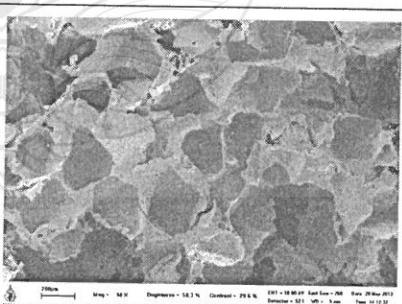


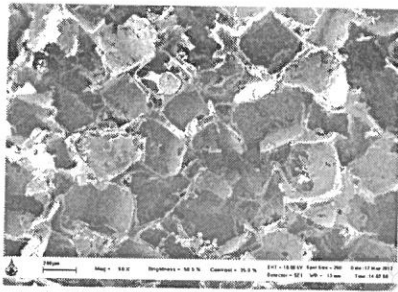
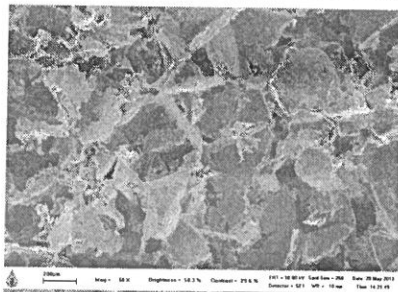
Figure 4.2 FTIR spectrum of sericin.

4.2 Morphological observation and pore size measurement

The combination of solvent casting and particulate leaching technique were used to fabricate porous scaffolds by using NaCl as the particulate leaching to generate porous structure. The effect of PLLA to NaCl ratio on the morphological appearance of both the neat and the GS-loaded PLLA-sericin hybrid scaffolds is shown in Table 4.1. The results showed that the interconnected porous structure of these scaffolds was obtained. The pore interconnectivity of the scaffolds increased with an increased in amount of NaCl. The pore size of the neat PLLA scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:8, 1:10, 1:1, and 1:15 was 330 ± 21 , 306 ± 22 , 300 ± 15 , and 290 ± 20 μm , respectively, while, the pore size of the GS-loaded PLLA-sericin hybrid scaffolds had been fabricated at PLLA:NaCl weight ratios of 1:8, 1:10, 1:12, and 1:15 was 344 ± 26 , 346 ± 25 , 341 ± 27 , and 343 ± 26 respectively.

Table 4.1 Selected images and pore sizes of the neat and the GS-loaded PLLA-sericin hybrid scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:8, 1:10, 1:12, 1:15

PLLA:NaCl ratios	Neat PLLA scaffolds	GS-loaded PLLA-sericin hybrid scaffolds
1:8		
Pore size (μm)	330±21	344±26
1:10		
Pore size (μm)	306±22	346±25
1:12		
Pore size (μm)	300±15	341±27

1:15		
Pore size (μm)	290±20	343±26

The scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:8 had the dense surface morphology with low pore interconnectivity. Therefore cells could not smoothly migrate into the inner scaffold. For the scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:12 and 1:15 had the appropriate surface morphology with high pore interconnectivity, so the cells could be easily transported deep in the scaffold. For use in bone tissue engineering applications, the scaffolds must possess a highly porous structure with an open fully interconnected geometry for providing a large surface area that will allow cell in-growth and uniform cell distribution. Average pore size, pore size distribution, pore volume, pore interconnectivity, pore shape, pore throat size, and pore wall roughness are also important parameters to consider for designing the scaffolds (Chen et al., 2001; Maurus et al., 2004).

4.3 Water retention 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 retention and weight loss behaviors of both the neat and the GS-loaded PLLA-sericin hybrid scaffolds after submersion in phosphate buffer solution for 24 and 48 h at 37°C were investigated and the results are shown in Figure 4.3 and 4.4. The water retention of the neat PLLA scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:8, 1:10, 1:12, and 1:15 after submersion for 24 h was

~136, ~234, ~325, and ~536%, respectively. At 48 h, the value increased to ~183, ~250, ~425, and ~598%, respectively. While the water retention of the GS-loaded PLLA-sericin hybrid scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:8, 1:10, 1:12, and 1:15 after submersion for 24 h was ~301, ~585, ~600, and ~855%, respectively. At 48 h, the value increased to ~362, ~653, ~706, and ~1,114, respectively.

The weight loss of the neat PLLA scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:8, 1:10, 1:12, and 1:15 after submersion for 24 h was 0.13, 0.63, 1.21, and 3.04%, respectively. At 48 h, the value increased to 0.57, 1.12, 2.48, and 5.21%, respectively. While the weight loss of the GS-loaded PLLA-sericin hybrid scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:8, 1:10, 1:12, and 1:15 after submersion for 24 h was 1.04, 2.22, 4.63, and 6.52% respectively. At 48 h, the value increased to 3.45, 4.32, 5.49, and 6.93%, respectively.

From Figure 4.3 and 4.4, the results showed that both the water retention and the weight loss increased with the increasing submersion time. In addition, both the water retention and the weight loss of these scaffolds increased with increasing the amount of NaCl in the PLLA scaffolds. Since increasing the amount of NaCl caused the porous structure of scaffolds to increase and water molecules can highly diffuse into the porous structure of scaffolds. The GS-loaded PLLA-sericin hybrid scaffolds showed the higher water retention and the weight loss than the neat PLLA scaffolds because sericin is a highly hydrophilic polymer with strong polar side chain such as hydroxyl, carboxyl and amino groups. Thus, the water molecules can highly diffuse and dissolve sericin out of the scaffolds. Moreover, the addition of GS into the scaffolds caused both the water retention and the weight loss to increase because GS molecules, which are good water soluble, can highly diffuse into the buffer solution. The maximum water retention and weight loss values after submersion time for 48 h were observed in the GS-loaded PLLA-sericin hybrid scaffolds that had been fabricated at PLLA:NaCl weight ratio of 1:15 because of the high porous structure of these scaffolds.

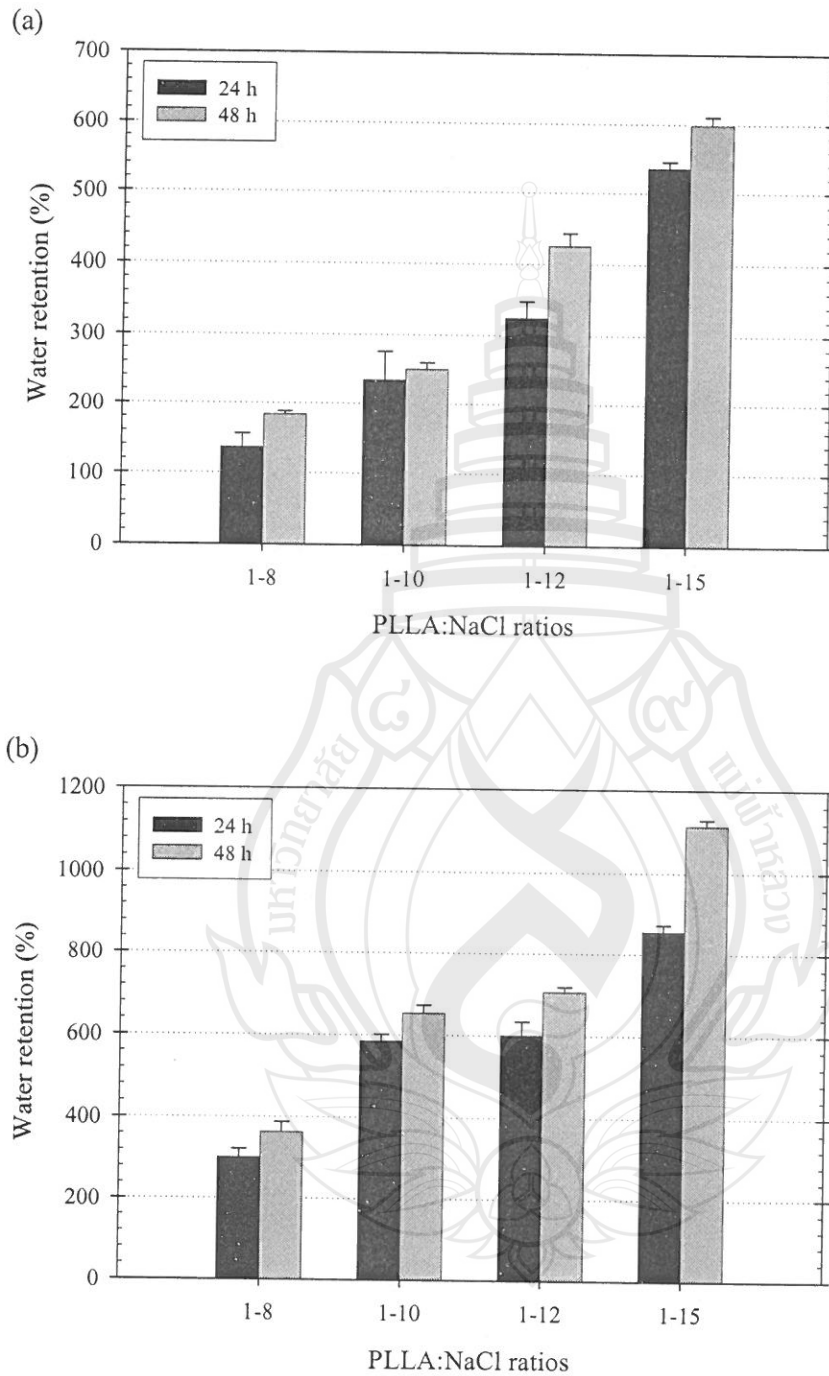


Figure 4.3 Water retention of (a) the neat and (b) the GS-loaded PLLA-sericin hybrid scaffolds in phosphate buffer solution for 24 and 48 h.

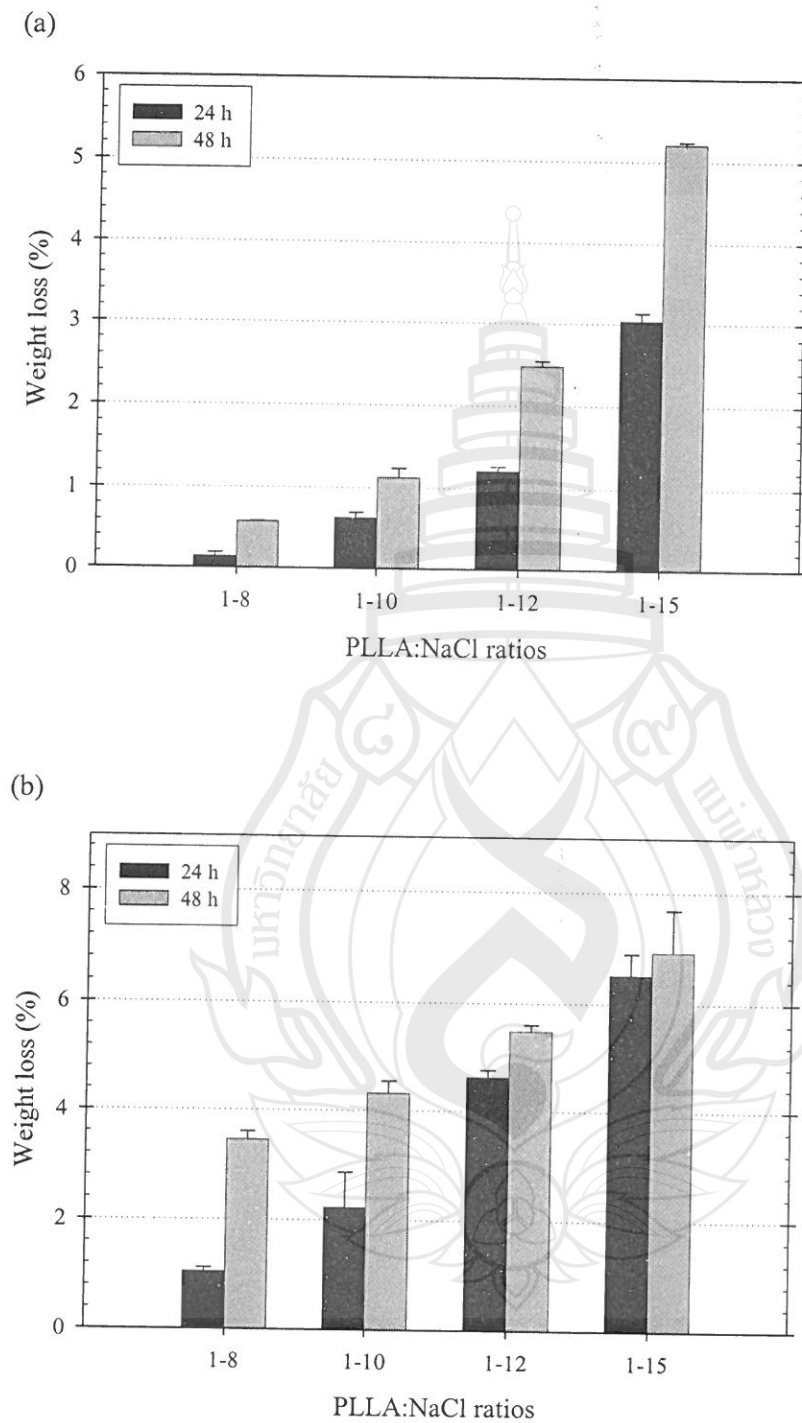


Figure 4.4 Weight loss of (a) the neat and (b) the GS-loaded PLLA-sericin hybrid scaffolds in phosphate buffer solution for 24 and 48 h.

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 culture with cells *in vitro* or implanted *in vivo*. Compressive test is widely accepted to evaluate the mechanical strength of tissue engineering scaffolds. 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. The compressive modulus of both the neat and the GS-loaded PLLA-sericin hybrid scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:8, 1:10, 1:12, and 1:15 is shown in Figure 4.5. The compressive modulus of the neat PLLA scaffolds had been fabricated at PLLA:NaCl weight ratios of 1:8, 1:10, 1:12, and 1:15 was ~1.83, ~1.02, ~0.68, and ~0.41, respectively, while, the compressive modulus of the GS-loaded PLLA-sericin hybrid scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:8, 1:10, 1:12, and 1:15 was ~1.26, ~0.74, ~0.32, and ~0.25, respectively. From these results, the increasing in the porous structure of the scaffolds with the increasing the NaCl content resulted in an observed decrease in the compressive modulus.

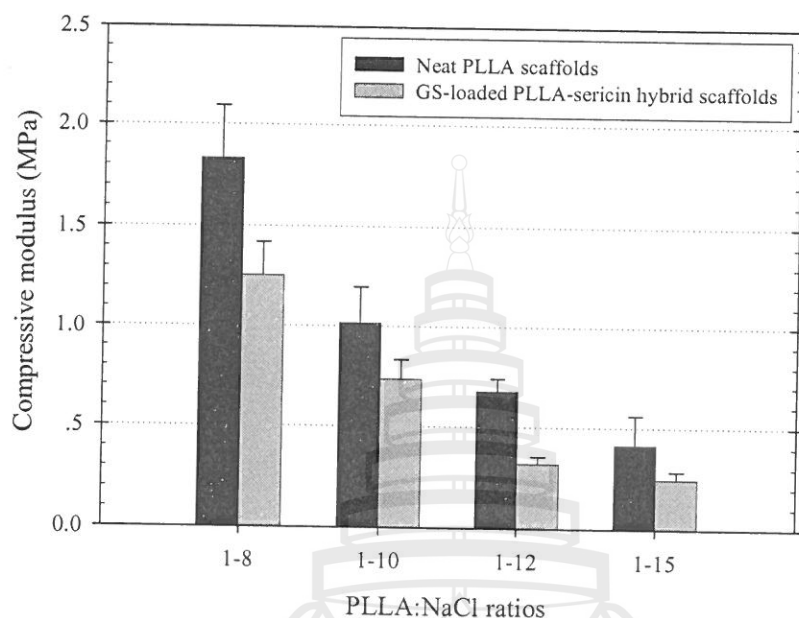


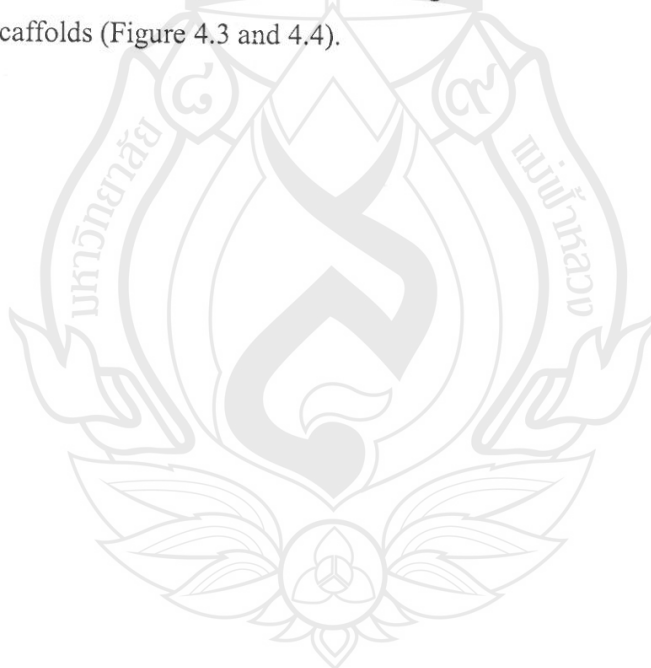
Figure 4.5 Compressive modulus of the neat and the GS-loaded PLLA-sericin hybrid scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:8, 1:10, 1:12, and 1:15 ($n = 5$).

4.5 Release of GS from GS-loaded PLLA-sericin hybrid scaffolds

The actual amounts of GS in the GS-loaded PLLA-sericin hybrid scaffolds were determined prior to investigating the release characteristics of GS from these samples, and it was found that the actual amounts of GS in the GS-loaded PLLA-sericin hybrid scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:8, 1:10, 1:12 and 1:15 were 78.4 ± 10.0 , 99.3 ± 1.7 , 97.3 ± 12.1 , and 105.4 ± 3.57 , respectively. These values were later used to calculate the cumulative amounts of GS released from the GS-loaded materials.

The release characteristics of GS from the GS-loaded PLLA-sericin hybrid scaffolds mats were investigated by the total immersion method over a period of time 2880 min in the phosphate buffer solution. The cumulative release profile of GS from the GS-loaded PLLA-sericin hybrid scaffolds were determined as the percentage corresponding to the weight of GS released divided by the actual weight of GS in the

sample (Figure 4.6). In all cases, the cumulative amounts of GS released into the buffer solution initially increased rapidly within the first 15 min, and more gradually increased afterwards. The cumulative amounts of GS released seemed to reach a plateau value at the end of the observational time period. The maximum amount of GS released from the GS-loaded PLLA-sericin hybrid scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:8, 1:10, 1:12 and 1:15 was ~24, ~32, ~36, and ~42%, respectively. The GS-loaded PLLA-sericin hybrid scaffolds that had been fabricated at PLLA:NaCl weight ratios of 1:15 showed the highest amount of GS released. The cumulative amounts of GS released were increased with increasing porous structure of samples. These results might be the high porous structure of samples could allow more penetration and diffusion of water into the polymer matrix enhancing the diffusion of GS out of the scaffolds. These results also could be explained by the results of the water retention and weight loss of the GS-loaded PLLA-sericin hybrid scaffolds (Figure 4.3 and 4.4).



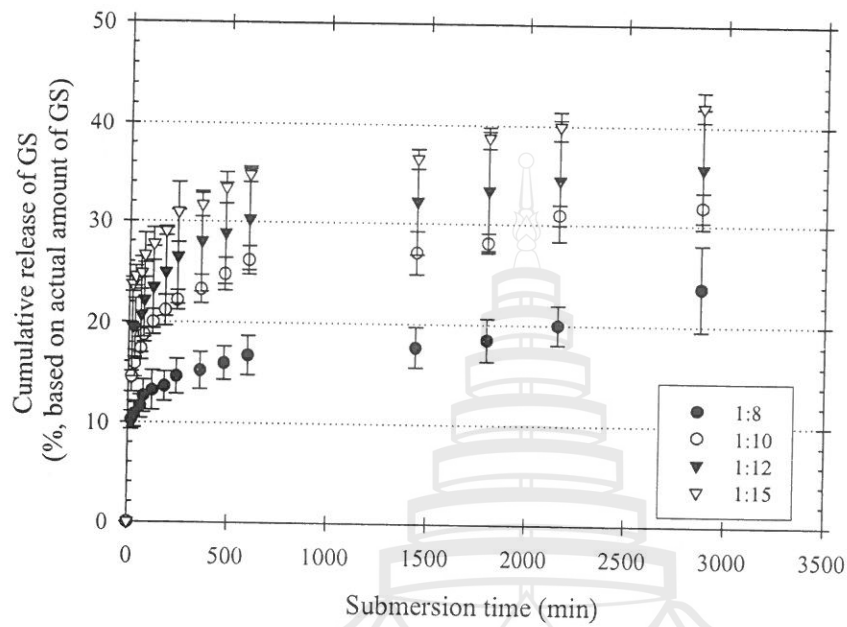


Figure 4.6 Cumulative release profile of GS from the GS-loaded PLLA-sericin hybrid scaffolds reported as the percentage corresponding to the weight of GS release divided by the actual weight of GS in the sample, as measured by the total immersion method in phosphate buffer at the physiological temperature of 37 °C.

4.6 Antibacterial evaluation of GS-loaded PLLA-sericin hybrid scaffolds

Two candidate bacteria (i.e., *E. coli* TISTR 780 as Gram-negative and *S. aureus* TISTR 1466 as Gram-positive) were used to evaluate the antibacterial activity of the GS-loaded PLLA-sericin hybrid scaffolds. The percentage of inhibition or colony count provides a quantitative procedure for evaluation of the antibacterial activity of the GS-loaded PLLA-sericin hybrid scaffolds and the results are shown in Table 4.2. According to these results, the neat PLLA scaffolds (i.e., control) showed no activity against the growth of both *E. coli* TISTR 780 and *S. aureus* TISTR 1466. All the GS-loaded PLLA-sericin hybrid scaffolds showed high activity against the growth of both *E. coli* TISTR 780 and *S. aureus* TISTR 1466 with percent reduction of 99.99 indicating these scaffolds could be used as bone tissue engineering scaffolds for treating osteomyelitis.

Table 4.2 Antibacterial activity of the GS-loaded PLLA-sericin hybrid scaffolds (n = 3).

Sample	Percent reduction (%R)
<i>E. coli</i> TISTR 780	
GS-loaded PLLA-sericin scaffolds 1:8	99.99%
GS-loaded PLLA-sericin scaffolds 1:10	99.99%
GS-loaded PLLA-sericin scaffolds 1:12	99.99%
GS-loaded PLLA-sericin scaffolds 1:15	99.99%
<i>S. aureus</i> TISTR 1466	
GS-loaded PLLA-sericin scaffolds 1:8	99.99%
GS-loaded PLLA-sericin scaffolds 1:10	99.99%
GS-loaded PLLA-sericin scaffolds 1:12	99.99%
GS-loaded PLLA-sericin scaffolds 1:15	99.99%

4.7 Indirect cytotoxicity evaluation of neat and GS-loaded PLLA-sericin hybrid scaffolds

To evaluate both the neat and the GS-loaded PLLA-sericin hybrid scaffolds could be used as bone scaffolds, indirect cytotoxicity evaluation was carried out on these scaffolds. Both the neat and the GS-loaded PLLA-sericin hybrid scaffolds were evaluated for their cytotoxicity. The viability of MC3T3-E1 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. Obviously, the viability of the cells cultured with all the extraction media from all the GS-loaded PLLA-sericin hybrid scaffolds ranged between ~84 and ~125%, indicating that all the GS-loaded PLLA-sericin hybrid scaffolds were proven non-toxic to MC3T3-E1 cells. Thus, these scaffolds have potential use for bone tissue engineering.

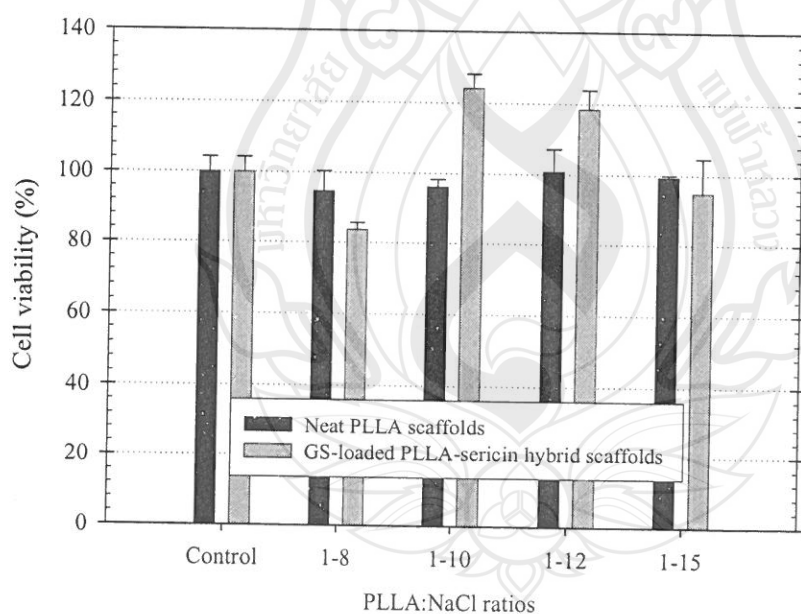


Figure 4.7 Indirect cytotoxicity evaluation of the neat and the GS-loaded PLLA-sericin hybrid scaffolds (n = 3).

CHAPTER 5

CONCLUSION

Both the neat and the GS-loaded PLLA-sericin hybrid scaffolds were successfully fabricated by particulate leaching and dipping methods. Sericin and GS were loaded into the neat PLLA scaffolds in order to prepare the GS-loaded PLLA-sericin hybrid scaffolds for use in bone tissue engineering. The effect of variation in the PLLA:NaCl weight ratios of 1:8, 1:10, 1:12 and 1:15 on the morphology of these scaffolds was studied. The pore size of the neat PLLA scaffolds ranged between 290 and 330 μm , while that of the GS-loaded PLLA-sericin hybrid scaffolds ranged between 341 and 346 μm . The pore interconnectivity of these scaffolds increased with an increased in the amount of NaCl. Both the neat and the GS-loaded PLLA-sericin hybrid scaffolds that had been fabricated at PLLA:NaCl weight ratio of 1:15 showed the high interconnected porous structure. Both the water retention and the weight loss increased with increasing NaCl content and submersion time. The increasing in the porous structure of the scaffolds with the increasing the NaCl content resulted in an observed decrease in the compressive properties. Moreover, the cumulative amounts of GS released were increased with increasing the porous structure of scaffolds. All the GS-loaded PLLA-sericin hybrid scaffolds showed high activity against the growth of both *E. coli* TISTR 780 and *S. aureus* TISTR 1466. Lastly, all the GS-loaded PLLA-sericin hybrid scaffolds were proven non-toxic to MC3T3-E1 cells, indicating their potential uses for bone tissue engineering.

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BIOGRAPHY

1. Principle investigator

Dr. Orawan Suwantong

Lecturer in School of Science, Mae Fah Luang University

Education

2004-2009: Ph.D. (Polymer Science), The Petroleum and Petrochemical College, Chulalongkorn University (an international program, in academic partnership with The University of Michigan, The University of Oklahoma, and Case Western Reserve University), Thailand

2000-2004: B.Sc. (Industrial Chemistry), Faculty of Science, Chiang Mai University, Thailand

Working Experience

2009-present: Lecturer in Materials Science, School of Science, Mae Fah Luang University, Thailand

2008: Research Assistant in Department of Chemical and Materials Engineering, The University of Alberta, Edmonton, Canada

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 Petrochemical 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 Petrochemical 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 Petrochemical 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 repellent products. Poster presentation at The 4th Research Symposium on Petrochemical 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.
10. **O. Suwantong**, P. Pankongadisak, S. Dechathai, and P. Supaphol. (2012, October 10-12) Electrospun poly(lactic acid) fiber mats containing herbal substances for drug delivery system and wound dressing applications. Poster presentation at การประชุมนักวิจัยรุ่นใหม่พบเมธีวิจัยอาวุโส สกว. ครั้งที่ 12, Phetchaburi, Thailand.
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
12. P. Pankongadisak, N. Warakorn, P. Supaphol, and **O. Suwantong**. (2012, October 17-19) Resveratrol-loaded gelatin films and their release characteristics. Poster presentation at The 38th Congress on Science and Technology of Thailand (STT38), Chiang Mai, Thailand
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2. Co-investigator

Boontharika Chuenjitkuntaworn, DDS., PhD.

Affiliation

Department of Oral Biology, Faculty of dentistry, Naresuan University, Phitsanulok, 65000, Thailand.

Education & Experience

2010-present Lecturer, Department of Oral Biology, Faculty of dentistry, Naresuan University, Phitsanulok, Thailand

2009 Ph.D. Oral biology, Faculty of dentistry, Chulalongkorn University, Bangkok, Thailand

2009 Oral presentation: *In vitro* and *in vivo* biological behavior of bone cells on polycaprolactone/hydroxyapatite scaffold., US-Japanese Symposium, Niigata, Japan

2008 Poster presentation: Differentiation of human dental pulp cells on polycaprolactone/hydroxyapatite scaffold., The IADR General Session and Exhibition, Toronto, Canada

2007 Poster presentation: Biological behavior of bone cells on Polycaprolactone/Hydroxyapatite scaffold., FDI World Dental Federation congress, Dubai, United Arab Emirates

2006 Poster presentation: Electrospun Polylactic Acid Fiber for Bone Regeneration., The IADR General Session and Exhibition, Brisbane, Australia
 2003 Staff, Oral biology department, Faculty of dentistry, Naresuan University, Phitsanulok, Thailand
 1997-2002 DDS. Faculty of dentistry, Mahidol University, Bangkok, Thailand

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P. Sangsanoh, S. Waleetorncheepsawat, O. Suwantong, P. Wutticharoenmongkol, O. Weeranantanapan, B. Chuenjitkuntaworn, P. Cheepsunthorn, P. Pavasant and P. Supaphol. "In Vitro Biocompatibility of Schwann Cells on Surfaces of Biocompatible Polymeric Electrospun Fibrous and Solution-Cast Film Scaffolds." Biomacromolecules, 2007; 8, 1587-1594.

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Research of interest:

1. Bone Tissue engineering, Bone regeneration in vitro and in vivo
2. Bioactive molecule induced bone mineralization such as BMP-2