In Vivo Bone Regeneration of Thai Silk Fibroin Scaffolds with Gelatin, Hydroxyapatite and Hyaluronic Acid

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Abstract

Three-dimensional Thai silk fibroin-based scaffolds have been developed and reported in our previous study on their appropriate morphology, physical properties and, in particular, promising potential to promote the growth of bone cells *in vitro*. Moreover, our previous study found that they were non-toxic to cells *in vivo*. In this work, three types of Thai silk fibroin-based scaffolds, including conjugated gelatin/Thai silk fibroin scaffold (CGSF), hydroxyapatite/conjugated gelatin/Thai silk fibroin scaffold (CGSF4) and hyaluronic acid/Thai silk fibroin scaffold (HSF), were investigated for their *in vivo* osteogenic potential in rat model. Each Thai silk fibroin-based scaffold was implanted in the bone defect (6 mm) on the radius bone of Wistar rats for 12 weeks. Bone regeneration was analyzed by micro-CT and semi-quantitative data evaluated from histological slides, compared to the control group (no implanted scaffold). The micro-CT result showed that the most pronounced new bone was noticed in the implant case of CGSF4 scaffold. The result of histopathologically semi-quantitative analysis showed that all scaffolds could enhance new bone formation. As a result, the Thai silk fibroin scaffold modified with gelatin conjugation and hydroxyapatite deposition (CGSF4) possessed great potential for being employed as bone scaffold for bone tissue engineering application.

Introduction

Bone fracture is a medical condition which can occur in people of all ages. Aging people, especially those suffering from osteopenia and osteoporotic, are more prone to bone fracture, particularly of the femur and hip. In case of multiple broken bones or open fracture, the removal of broken bones is required to prevent serious infection, creating a bone gap. Bone replacement or bone graft is often required to facilitate healing of large bone gap, promoting bone regeneration and repair (Langer and Vacanti, 1993). However, there are some drawbacks and limitations of the usage of typical bone grafts in clinical practice; therefore, synthetic bone graft is produced and employed as scaffolds for bone tissue engineering (Drosse et al., 2008; Doron and Amy, 2002; Laurencin et al., 2006). Naturally occurring materials, such as fibroin, collagen, gelatin and hyaluronic acid, were of interest to be used as scaffolds due to their biocompatible, biodegradable and non-toxic characteristics (Peter, 2004; Hutmacher, 2000; Stevens, 2008; Nukavarapu et al., 2011).

Silk fibroin produced from mulberry silk worm (*Bombyx mori*) is the major component in insoluble silk fiber, about 75-80 wt%. Silk fibroin can be used as scaffolds in various forms such as porous three-dimensional structure, nanofiber and hydrogel due to their great mechanical property, biocompatibility and slow degradation (Melke et al., 2016; Inoue et al., 2000). Silk fibroin nanofibrous membrane was reported to have excellent biocompatibility and promote bone healing with complete defect coverage after 8 weeks of *in vivo* implantation in rabbit calvarial defects (Kim et al., 2005). Thai silk, a type of mulberry silks markedly appeared as yellow cocoon, contains more silk gum (sericin) than other types of mulberry silks (Ministry of Argiculture and Cooperatives, 2009). Thai silk fibroin was firstly reported as a scaffold and introduced to conjugate with gelatin, a collagen-derived protein, to enhance its biological properties in bone tissue engineering (Chamchongkaset et al., 2008; Vachiraroj et al., 2009).

In 2010, Tungtasana et al. (2010) reported *in vivo* tissue response and biodegradation of four Thai silk fibroin-based scaffolds incorporated with gelatin and hydroxyapatite. The scaffolds were implanted into the subcutis of Wistar rats, according to ISO10993-6: Biological Evaluation of Medical Devices. After 12 weeks of implantation, all scaffolds were evaluated and classified as "non-irritant" to "slight-irritant", compared to Gelfoam® (control sample). Moreover, the hydroxyapatite/conjugated gelatin/Thai silk fibroin scaffold (CGSF4) was also tested for its safety by Thailand Institute of Scientific and Technological Research, including systemic injection test, acute dermal irritation test, skin sensitization test and cytotoxicity test. The results showed that CGSF4 scaffold was not toxic to animals and did not exhibit adverse effects on the skin. Taken together, CGSF4 scaffold showed high potential for further development in bone tissue engineering.

Another material widely used for medical purpose due to its great biocompatibility is hyaluronic acid (HA), a glycosaminoglycan present in tissue and extracellular matrix of musculoskeletal (Fraser and Laurent, 1989). HA hydrogels were shown to support the attachment and proliferation of MC3T3-E1 cells in an *in vitro* study (Cui et al., 2015). Silk fibroin/hyaluronic acid scaffold was reported to promote cell viability, attachment and migration of neural stem cells of Sprague Dawley rats (Ren et al., 2009) and promote cell growth of stem cells and new bone formation (Garcia-Fuentes et al., 2009). In addition, there was a report on the effect of HA to support *in vivo* osteoinductivity. Implanted HA or bone graft with combined HA induced new bone formation via osteoblast differentiation process, and filled the bone gap in at a shorter time compared to no implanted HA group (Sasaki and Watanabe, 1995). New bone formation, angiogenesis and connective tissue formation were significantly higher in the graft and graft with combined HA groups (Diker et al., 2015).

In this study, the *in vivo* osteogenic potential of three-dimensional Thai silk fibroin-based scaffolds was examined. Two types of scaffold were selected from our previous studies, Thai silk fibroin scaffold conjugated with gelatin (CGSF) and Thai silk fibroin scaffold conjugated with gelatin and deposited with hydroxyapatite (CGSF4). The other scaffold was Thai silk fibroin scaffold coated with hyaluronic acid (HSF). A critical bone defect model on the radius of Wistar rat was employed to investigate new bone formation, compared to a blank bone defect (sham).

Materials and Methods

Materials: *Bombyx mori* Thai silk cocoons (Nangnoi-Srisaket 1 race) were kindly supplied by Queen Sirikit Sericulture Center, Nakhonratchasima province, Thailand. Type A gelatin was supplied by Nitta Gelatin Inc., Osaka, Japan. Hyaluronic acid (viscosity-averaged molecular weight of 722,000 Da) was purchased from Namsiang Internatonal CO., LTD., Bangkok, Thailand. Other chemicals used were analytical grade.

Preparation of Thai silk fibroin-based scaffolds
Preparation of Thai silk fibroin scaffold: Thai silk fibroin (SF) solution and scaffold were prepared according to the method previously described by Kim et al. (2005). In brief, silk sericin was removed from the cocoons by boiling in 0.02 M sodium carbonate (Na_2Co_3) solution and rinsing with deionized water. The degummed SF was dissolved in 9.3 M lithium bromide (LiBr) solution at 60°C. The solution was dialyzed against DI water for 2 days to form 6-6.5 wt% solution. SF scaffolds were fabricated using salt-leaching technique by adding sodium chloride crystals, with the size of 600-710 microns, into the SF solution contained in cylinder-shaped containers. The containers were left at room temperature until SF became gel. After that salt crystals were leached out by deionized water and Thai silk fibroin scaffolds were obtained by air drying.

Preparation of Thai silk fibroin scaffold conjugated with gelatin (CGSF): Thai silk fibroin scaffolds

conjugated with gelatin were prepared following our previous reports (Chamchongkaset et al., 2008; Tungtasana et al., 2010). In brief, the SF scaffolds were soaked in 0.5 wt% gelatin solution under vacuum for 2 h. After freeze-drying, the gelatin-coated silk fibroin scaffolds were dehydrothermal crosslinked at 140°C for 48 h under vacuum and further conjugated by immersing in a solution of 14 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 5.5 mM N-hydroxy-succinimide (NHS) for 2 h. Then, excess EDC and NHS were removed by rinsing the conjugated scaffolds with deionized water. CGSF scaffolds were obtained after air drying at room temperature and increased weight of the scaffolds after gelatin conjugation was determined and considered as the content of gelatin in the scaffold.

Preparation of Thai silk fibroin scaffold conjugated with gelatin and deposited with hydroxyapatite (CGSF4): Thai silk fibroin scaffolds conjugated with gelatin and deposited with hydroxyapatite were prepared following our previous report (Tungtasana et al., 2010). In brief, hydroxyapatite was deposited on the CGSF scaffold by an alternate soaking method. The scaffolds were soaked in 0.2 M $CaCl_2$ solution for 20 min and then transferred to 0.12 M Na_2HPO_4 solution for another 20 min, followed by rinsing in deionized water. This alternate soaking cycle was repeated 4 times. After air drying, CGSF4 scaffolds were obtained. Increased weight of the scaffolds after alternate soaking was considered as the content of deposited hydroxyapatite in the scaffold.

Preparation of Thai silk fibroin scaffold coated with hyaluronic acid (HSF): HSF scaffolds were prepared by immersing the SF scaffolds in 1 wt% hyaluronic acid solution under vacuum for 2 h prior to freeze-drying to allow the coating of hyaluronic acid onto the surface of SF scaffold. Similarly, increased weight of the scaffolds after coating was considered as the content of hyaluronic acid in the scaffold.

Physical characterization of Thai silk fibroin-based scaffolds: The scaffolds were sputter-coated with gold prior to observation of their morphology using a scanning electron microscope (SEM, JSM-5410LV, JEOL Ltd., Japan). Pore size of each scaffold was determined from one hundred random pores using SemAfore 5.21 software.

In vivo bone regeneration of scaffolds in rat model
Bone defect experiment: *In vivo* experiment was performed under the approval of the Ethics Committee of the Faculty of Veterinary Science, Chulalongkorn University (No. 1431073). A bone defect model on the left radius of female Wistar rats (12 weeks old, 200-300 g) was prepared according to the procedure reported by Ratanavaraporn et al. (2012). To evaluate the osteogenesis capability of the three scaffolds, the scaffolds, 2 mm in width and 6 mm in length, were sterilized by ethylene oxide and implanted into the bone defect of Wistar rats. No scaffolds were implanted in the control group.

Three rats were employed for each sample group. The rats were anaesthetized by inhalation of

Isoflurane. Forearm skin of the rats was shaved and disinfected by betadine solution and 70% ethanol. Then, the forearm skin and periosteum were longitudinally incised to approach the radius bone. A sharp defect (6 mm in length) was created at the mid diaphysis shaft of the radius bone by using an oscillating saw. The scaffold was implanted into the defect then the wound was closed with a suture. The rats were provided *ad libitum* access to feed and water and taken care in accordance with the institute's standard protocol for laboratory animal.

Radiography and micro-computed tomography (μ-CT) analysis: After the implantation, radiography was performed to investigate the excision area and position of the bone defect. Bone regeneration at the defect site was evaluated using micro-computed tomography (Skyscan 1173, Bruker Company, Belgium) at the energy of 80 kV, the current of 100 μA and the exposure time of 1110 ms. After 12 weeks of implantation, the animals were sacrificed with an overdose of CO_2. The left forearm of the rats was collected and fixed with 10% buffered formalin for 3 days and rinsed through phosphate buffer saline (PBS) before the scanning. Two-dimensional data were reconstructed by NRecon software (Skyscan, Belgium) to produce 3-dimensional images. Results were compared with the control group (no implanted scaffold).

Bone mineral density (BMD) of the radius bone was evaluated from cross-sectional images of the 3-dimensional radius bone using SkyScan CT-analyser program (Skyscan, Belgium). Percentage of BMD was calculated from the ratio of BMD of the new bone formation of proximal site of bone defect to BMD of the normal bone (6 mm upper and lower from implantation site) in each rat by the following equation.

$$\%BMD = \frac{\text{Bone mineral density of new bone}}{\text{Bone mineral density of normal bone}} \times 100$$

Moreover, %bone volume (bone volume/tissue volume (BV/TV)) was evaluated using SkyScan CT-analyser program and used to quantitatively compare the volume of mineralized bone per unit volume in each group.

Histological observation: When the micro-CT scanning finished, the radius bone and adjacent tissues were re-fixed in 10% buffered formalin for 3 days and then decalcified in 5% nitric acid solution for 3 more days. The specimens were paraffin-embedded and sectioned into 2 μm thickness before staining with hematoxylin and eosin (H&E) for histological evaluation under a light microscope. The bone sections were evaluated by a pathologist as blind samples.

Results

Physical characterization of Thai silk fibroin-based scaffolds: Figure 1 shows the cross-sectional morphology of Thai silk fibroin-based scaffolds. Pore size and % weight content of components in all scaffolds were summarized in Table 1. The morphology of the CGSF and HSF scaffolds showed

smooth surface and interconnected porous network with the pore size of 351±65 μm and 235±63 μm, as shown in Figures 1a and 1c, respectively. In contrast, porous structure with rough surface of the CGSF4 scaffold was noticed because of the deposited hydroxyapatite crystal on the surface as imaged in Figure 1b. The pore size of the CGSF4 scaffold was smaller than that of the CGSF scaffold.

In vivo bone regeneration of scaffolds: The bone defect at the incision site, demonstrated by the radiography

after the implantation (Figure 2), showed a sharp incision line. The new bone formation was observed by the micro-CT 3D technique. The results indicated that a new spike-shaped bone was found from the proximal end of the bones in all implanted groups. Interestingly, new bone formation was observed in the middle part of the defects in all scaffold-implanted groups at 12 weeks post implantation (Figure 3), but these findings were not seen in the control group.

Table 1   % Weight content of components and pore size of Thai silk fibroin scaffold conjugated with gelatin (CGSF), Thai silk fibroin scaffold conjugated with gelatin and deposited with hydroxyapatite (CGSF4), and Thai silk fibroin scaffold coated with hyaluronic acid (HSF)

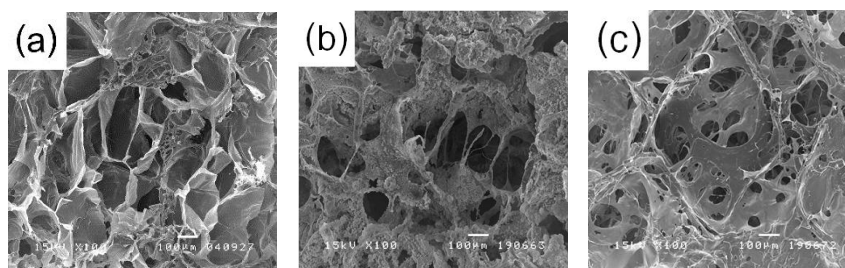| Properties	CGSF scaffold	CGSF4 scaffold	HSF scaffold
% weight content of components	Thai silk fibroin 92.45% Gelatin 7.55%	Thai silk fibroin 56.36% Gelatin 3.88% Hydroxyapatite 39.76%	Thai silk fibroin 78.03% Hyaluronic acid 21.97%
Pore size	351±65 μm	242±45 μm	235±63 μm

Figure 1   Scanning electron micrographs of (a) Thai silk fibroin scaffold conjugated with gelatin (CGSF), (b) Thai silk fibroin scaffold conjugated with gelatin and deposited with hydroxyapatite (CGSF4), and (c) Thai silk fibroin scaffold coated with hyaluronic acid (HSF). (Scale bar = 100 mm)

Figure 2   An x-ray image of a bone defect site in the middle of radius bone of a Wistar rat after implantation

The osteogenic potential of all Thai silk fibroin-based scaffolds in Wistar rats was evaluated via %bone mineral density (%BMD) and %bone volume as shown in Table 2. The highest %BMD and %bone volume were noticed in the case of implanted CGSF4 scaffold up to 66.44% and 77.81%, respectively. Remarkably, the longest length and the highest %BMD of new bone formed in the middle of defect site were noticed in the group with CGSF4 scaffold. In contrast, %BMD of new bone in the middle of CGSF scaffold and HSF scaffold could not be quantitatively evaluated because the newly formed bone was too small.

Histological results: Microscopically, the bone healing was seen in all scaffold-implanted groups at 12 weeks post implantation (Figure 4) characterized by the presence of callus formation, new bone trabeculae formation, fibrosis, neovascularization, and the

infiltration of osteoblast- and osteoclast-like cells at the implanted area. The callus was observed mainly at the periosteal area (Figure 4a). The new bone trabeculae formation with the fibrosis and neovascularization (Figures 4b and 4c) commingled. The remains of implanted scaffolds was noted in all scaffold-implanted groups (Figure 4a). However, inflammatory cells such as neutrophil, lymphocyte, macrophage and giant cell were not observed around the defect sites. The semi-quantitative analysis of bone healing in each group was summarized in Table 3. The semi-quantitative analysis revealed the highest amount of osteoblast infiltration, fibrous tissue, neovascularization and callus formation in the CGSF and HSF scaffolds. In addition, the lowest sign of bone healing markers was noticed in the CGSF4 scaffold. This was in contrast to the micro-CT results, possibly due to some discrepancies in sectioning leading to an

imperfect field of collected sample. In the control group, histological result could not be obtained because the defect site was empty.
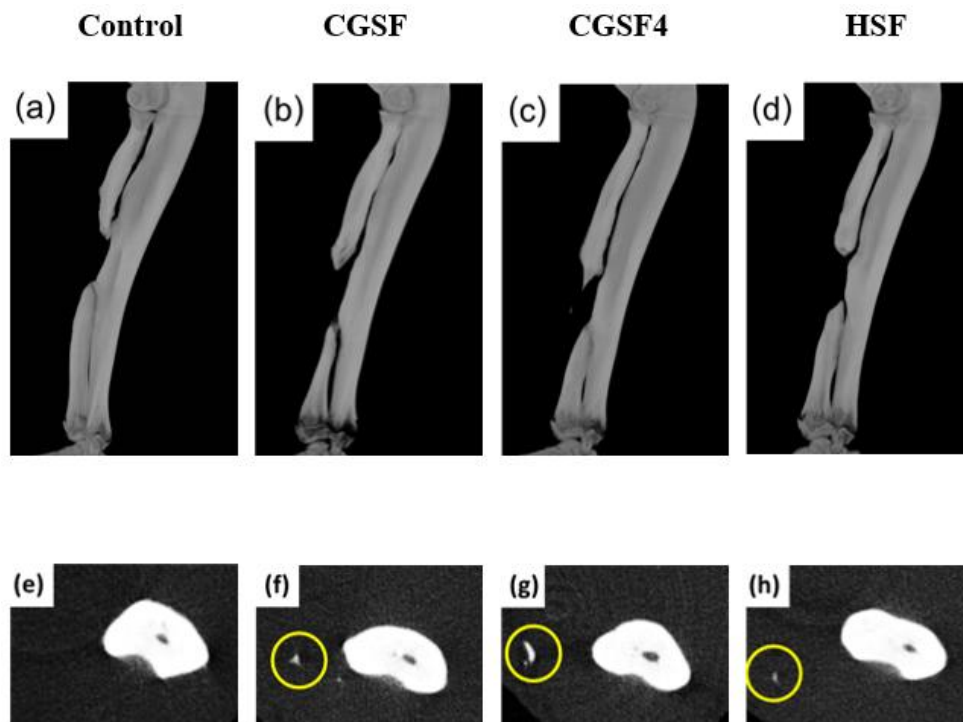

Figure 3 Micro-CT 3D images (a-d) and cross-sectional images (e-h) of bone defect sites with and without implanted scaffolds after 12 weeks of implantation. (a, e) control group, (b, f) Thai silk fibroin scaffold conjugated with gelatin (CGSF), (c, g) Thai silk fibroin scaffold conjugated with gelatin and deposited with hydroxyapatite (CGSF4) and (d, h) Thai silk fibroin scaffold coated with hyaluronic acid (HSF). The circles indicate new bone formed in the middle of the defects.
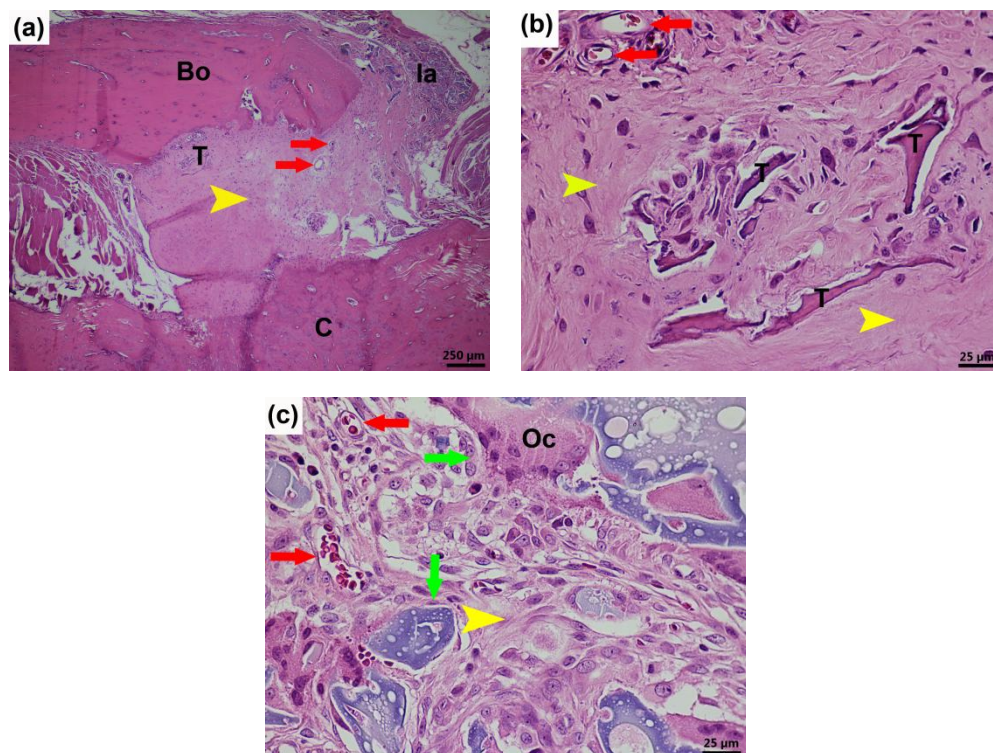

Figure 4 Bone sections of CGSF scaffold at 4x (a) and 40x (b), indicating region of implant scaffold and radius bone, callus formation and bone trabecular. Hematoxylin and eosin stained sections of CGSF scaffold at 40x (c), indicating tissue reaction of implant scaffold, neovascularization, fibrous tissue, osteoblast and osteoclast. (Bo = normal radius bone, Ia = implant material, C = callus formation, T = bone trabecular, Yellow arrow = fibrous tissue, green arrow = osteoblast, and red arrow = neovascularization)

Table 2 %BMD and %bone volume of new bone formed in bone defects of Wistar rats after implantation with Thai silk fibroin scaffold conjugated with gelatin (CGSF), Thai silk fibroin scaffold conjugated with gelatin and deposited with hydroxyapatite (CGSF4), and Thai silk fibroin scaffold coated with hyaluronic acid (HSF) for 12 weeks, compared to control group

Analysis	Control	CGSF	CGSF4	HSF
%BMD of new bone formed at proximal side	51.95%	19.10%	66.44%	40.72%
%Bone volume	77.20%	65.13%	77.81%	55.61%
Length of new bone formed in the middle of scaffold (mm)	-	0.233	0.909	0.639
%BMD of new bone formed in the middle of scaffold	-	Cannot be calculated	120.8%	Cannot be calculated

Table 3 Semi-quantitative analysis of new bone formation in the groups with implanted scaffolds (CGSF, CGSF4, and HSF scaffolds) compared to the control group

Scaffolds	Osteoblast infiltration	Fibrosis	Neovascularization	Callus formation
Control	ND	ND	ND	ND
CGSF	++	+++	+++	+++
CGSF4	+	+	+	+
HSF	++	+++	++	+++

Note: ND = not determined, + = mild (about 1-30% of examined field), ++ = moderate (about 31-70% of examined field), and +++ = marked (about 71-100% of examined field)

Discussion

The potential of three-dimensional Thai silk fibroin-based scaffolds on *in vivo* bone regeneration of Wistar rat was evaluated by implanting the scaffolds in the radius bone defect and comparing to the control group. From the micro-CT analysis after 12 weeks of implantation, all Thai silk fibroin-based scaffolds, without any cells and growth factors, were found to be able to induce new bone formation. Newly formed bone was observed in the middle part of the defects in all scaffold-implanted groups, but the findings were not noticed in the control group. This indicates the significant potential of all Thai silk fibroin-based scaffolds for *in vivo* bone regeneration. A similar observation was reported by Harada et al. (2014) on the new bone formation in the middle of femur bone defect of Fischer rat found in bared PLGA scaffold at 4 weeks after implantation.

The results on micro-CT analysis revealed that the implanted scaffolds into the defect sites could recruit bone cells and support bone growth. It should be noted that these scaffolds released sufficient biological properties without any growth factors incorporated. In particular, among the three types of Thai silk fibroin-based scaffolds, Thai silk fibroin scaffold conjugated with gelatin and deposited with hydroxyapatite (or CGSF4 scaffold) indicated relatively high degree of osteogenesis compared to the other two scaffolds, as noticed from the highest %BMD, %bone volume and the most obvious occurrence of new bone in the middle of scaffolds.

The incorporation of gelatin and hydroxyapatite obviously played an important role in promoting the biological properties of Thai silk fibroin scaffold. The presence of gelatin and hydroxyapatite in Thai silk fibroin scaffold was shown to enhance the proliferation and differentiation of Mouse osteoblast-like cells (MC3T3) *in vitro* in our previous works (Chamchongkaset et al., 2008; Vachiraroj et al., 2009). The presence of higher gelatin content in a scaffold was also reported to improve cell adhesion and proliferation (Zhang et al., 2011). The new bone formation *in vivo* reported by Kasuya et al. (2012) also showed that new bone area increased from 4.7 to 5.2 fold in calcium phosphate cement and gelatin blended scaffold implanted group, compared to pure calcium phosphate cement scaffold implanted group. Hydroxyapatite presented in other scaffolds could enhance osteoconduction and bone regeneration as reported by Chang et al. (2000) and Kaito et al. (2006). Moreover, the increased quantity of hydroxyapatite in biomimetic collagen-hydroxyapatite scaffold was reported to be related to the increase in new bone formation and calcium deposition in Wistar rat model (Gleeson et al., 2010).

Together with our reports on appropriate physical properties, nontoxicity and safety of CGSF4 scaffold published earlier (Tungtasana et al., 2010), it can be concluded that Thai silk fibroin scaffold conjugated with gelatin and deposited with hydroxyapatite (CGSF4) has excellent potential for being employed as a scaffold for bone tissue engineering.

Acknowledgements

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บทคัดย่อ

ความสามารถในการสร้างกระดูกในสัตว์ทดลองของโครงเลี้ยงเซลล์ที่ผลิตจากไฟโบรอินไหมไทย เจลาติน ไฮดรอกซีอะพาไทต์ และกรดไฮยาลูรอนิค

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โครงเลี้ยงเซลล์สามมิติที่มีไฟโบรอินไหมไทยเป็นองค์ประกอบหลักได้รับการพัฒนาและรายงานในงานวิจัยก่อนหน้านี้ ถึงโครงสร้างสัณฐาน สมบัติทางกายภาพ และความสามารถในการส่งเสริมการเจริญเติบโตของเซลล์กระดูกในระดับห้องปฏิบัติการ นอกจากนี้โครงเลี้ยงเซลล์เหล่านี้ยังไม่เป็นพิษต่อเซลล์ในระดับสัตว์ทดลองอีกด้วย งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาความสามารถในการสร้างเนื้อเยื่อกระดูกในหนูทดลองของโครงเลี้ยงเซลล์ที่มีไฟโบรอินไหมไทยเป็นองค์ประกอบหลัก 3 ชนิด ได้แก่ โครงเลี้ยงเซลล์ไฟโบรอินไหมไทยที่เตรียมด้วยวิธีกำจัดเกลือออกและปรับปรุงพื้นผิวด้วยการคอนจูเกตกับเจลาติน (CGSF) โครงเลี้ยงเซลล์ไฟโบรอินไหมไทยที่เตรียมด้วยวิธีกำจัดเกลือออกและปรับปรุงพื้นผิวด้วยการคอนจูเกตกับเจลาตินและการสะสมไฮดรอกซีอะพาไทต์ (CGSF4) และโครงเลี้ยงเซลล์ไฟโบรอินไหมไทยที่เตรียมด้วยวิธีกำจัดเกลือออกและปรับปรุงพื้นผิวด้วยการเคลือบกรดไฮยาลูรอนิค (HSF) โครงเลี้ยงเซลล์แต่ละชนิดถูกฝังลงแทนที่ช่องว่างของกระดูก (ยาว 6 มิลลิเมตร) บนกระดูกเรเดียส (Radius) ของหนูวิสต้าเป็นเวลา 12 สัปดาห์ จากนั้นประเมินความสามารถของโครงเลี้ยงเซลล์ในการสร้างเนื้อเยื่อกระดูกด้วยการถ่ายภาพด้วยเครื่องเอ็กซเรย์คอมพิวเตอร์ระดับไมโคร (micro-CT) และการวิเคราะห์ทางจุลพยาธิ รวมถึงการวิเคราะห์เชิงกึ่งปริมาณบริเวณช่องว่างของกระดูกโดยนักพยาธิวิทยา โดยเปรียบเทียบผลการประเมินกับกลุ่มควบคุมที่ไม่มีการฝังโครงเลี้ยงเซลล์ ผลการศึกษาด้วยการถ่ายภาพด้วยเครื่องเอ็กซเรย์คอมพิวเตอร์ระดับไมโคร พบการสร้างเนื้อเยื่อกระดูกใหม่มากที่สุดในหนูทดลองกลุ่มที่ฝังโครงเลี้ยงเซลล์ไฟโบรอินไหมไทยที่เตรียมด้วยวิธีกำจัดเกลือออกและปรับปรุงพื้นผิวด้วยการคอนจูเกตกับเจลาตินและการสะสมไฮดรอกซีอะพาไทต์ ผลการวิเคราะห์ทางจุลพยาธิในเชิงกึ่งปริมาณ พบว่า โครงเลี้ยงเซลล์ทุกชนิดสามารถส่งเสริมการสร้างเนื้อเยื่อกระดูกใหม่ได้ ผลการศึกษานี้แสดงให้เห็นว่าโครงเลี้ยงเซลล์ไฟโบรอินไหมไทยที่เตรียมด้วยวิธีกำจัดเกลือออกและปรับปรุงพื้นผิวด้วยการคอนจูเกตกับเจลาตินและการสะสมไฮดรอกซีอะพาไทต์ (CGSF4) มีศักยภาพสูงในการใช้เป็นโครงเลี้ยงเซลล์เพื่องานวิศวกรรมเนื้อเยื่อกระดูกต่อไป

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