Effect of Thai Silk Fibroin on Mechanical Properties of Bioactive Glass Silk Fibroin Hybrid Bone Scaffolds

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Abstract. Bioactive glass (70 mol% Si and 30% Ca; 70S30C) has been used as tissue engineering scaffolds in various research despite its brittleness due to its easy-to-replicate formula, extensive interconnected pores and natural bone bonding property of bioactive glass (BG). Thai silk fibroin (SF) is natural polymer with many desirable properties; biocompatibility, mechanical strength, biodegradability. Using BG-SF hybrid scaffolds could employ the positive effects of both bioactive glass and silk fibroin. Serving as bone scaffolds, BG-SF scaffolds must resist compression and other types of mechanical load depending on the application. This study fabricates scaffolds with various ratios of BG:SF (BG:SF; 90:10, 80:20 with 100:0 as control), and their stress-strain profiles were investigated. As expected, the scaffolds with highest BG content exhibited the curve resembling that of porous foam while the elastomeric stress-strain profile was becoming more evident when the SF content was increased. This study hence demonstrated fine-tuning mechanical properties of the BG using locally enriched SF.

1. Introduction

Bioactive glass (BG) has been widely used in tissue engineering research. This is due to its composition similarity to natural bone minerals, ability to bond with newly formed bone, and the release of ions promoting osteogenesis [1-3]. There are many formulae of BG and numerous additives can be added to enhance certain aspects to suit different applications [4, 5]. 70S30C is one of the easiest formulae to fabricate. It consists of only 2 distinct compositions: silica and calcium (70% mol being silica and 30% mol being calcium) [2, 6, 7]. However, being ceramic in nature leads to having a brittle structure.

Silk fibroin (SF) is also a biomaterial widely used, both in research and clinics. The SF is strong, non-inflammable, and biodegradable. These properties make SF an excellent candidate for various approaches in tissue engineering [8]. In this study, to overcome the brittleness of BG, SF is added to reinforce the silica network formed beforehand to create a more resilient scaffold overall. SF can be crosslinked with BG by (3-glycidoxypropyl)trimethoxysilane (GPTMS) [9]. The resulting hybrid scaffolds were reported to have enhanced mechanical stability. Briefly, GPTMS is a silane coupling agent with epoxy and methoxysilane group with oxirane ring reacting to amine group forming covalent bond. Hydrolysis of methoxy resulted in pendant silanol groups which condense into Si-O-Si.

70S30C BG usually requires a silica network with calcium incorporated. There are many fabrication methods. In this study, the gel foaming method is used. Gel foaming can be done easily and does not
require any specialized equipment [2]. In the final step, the solution containing every composition of the scaffold is agitated vigorously, introducing air bubbles, and inducing gelation. These bubbles will later act as porogens and create an interconnected porous scaffold [10] which is an advantageous property for the scaffold. The porous scaffolds enable cells to communicate, proliferate and differentiate within the scaffolds, ensuring maximum cellular activities [11]. However, porous scaffolds often face similar pitfall: structural integrity. It is a challenge to navigate this limitation while providing adequate porosity for the cells to function properly.

This study focused on the effect the of ratio between BG and SF on the integrity of the scaffolds. Hybrid scaffolds with various ratios of BG:SF were fabricated by gel foaming method. The scaffolds were then characterized.

2. Experimental methods

2.1. Materials

Silk cocoons were obtained from The Queen Sirikit Department of Sericulture, Thailand. Tetraethyl orthosilicate (TEOS), lithium bromide (LiBr), n-hexane, and Teepol were purchased from Sigma-Aldrich. Hydrochloric acid (HCl) and Hydrofluoric acid (HF) were purchased from QreC. Calcium chloride (CaCl₂) and sodium carbonate (Na₂CO₃) were purchased from Ajax Finechem and GPTMS was purchased from Gelest, Inc.

2.2. Preparation of silk fibroin solution

Silk cocoons were dissolved in 9.3 M LiBr solution, but they must first be washed and degummed. The process was described elsewhere [12]. Briefly, silk cocoons were washed with deionized water to clean out debris and larvae. Then, 0.02 M Na₂CO₃ solution was used to boil the cocoon for 20 minutes, this was to remove the glue-like coating called sericin, or degumming [13]. The cocoons were then boiled for the second time after washing with deionized water. After degumming, the fibres were left to dry open-air for 2 days. The dried fibres were then dissolved in 9.3 M LiBr solution and dialyzed for 3 days in deionized water. The solution was then centrifuged for 20 minutes at 9000 rpm, 4°C to remove remaining debris.

2.3. Scaffold fabrication

TEOS was hydrolysed by HCl to and allow to condense to form silica network. Calcium chloride was added, as a network modifier, with molar ratio of Si:Ca of 7:3. SF solution was added last with varying ratios of BG: SF. SF solution was preconditioned by GPTMS before adding to the sol. After mixing all the ingredients, the sol was agitated vigorously with milk froth whipper to induce gelation and introduce air bubble. The sol was agitated until foamed and then transferred to a silicone mould. The foam was left to age for 3 days at 60°C with the cover closed. Later, the cover was removed, and the foam was left to fully dry for another 2 days at 60°C. The scaffolds were then collected and stored in a dry environment at room temperature [2, 6].

3. Characterizations

3.1. Thermogravimetric analysis

The scaffolds’ organic/inorganic mass ratios and thermal stability were investigated with thermogravimetric analysis. The scaffolds were heated from room temperature to 900°C with a heating rate of 20°C/min under a nitrogen atmosphere.

3.2. Surface morphology

Scaffolds were subjected to scanning electron microscope (SEM) with Hitachi SU3500 model to observe scaffold morphology. The scaffolds were gold-coated, and the voltage was 5kV.
3.3. Porosity
To determine the porosity of each scaffold, fluid displacement analysis was conducted. We used n-hexane as the fluid that filled the porous structure [10]. Briefly, the scaffolds were cut into a 5×5×5 mm cubes. The cubic scaffolds were submerged under n-hexane in a closed container and weighted again. The scaffolds were left to rest for 5 minutes and retrieved from the container. The containers were weighed again and calculated using the following equation to determine the amount of n-hexane that occupied the porous cavity:

\[
\% \text{ Porosity} = \left( \frac{W_1 - W_3}{W_2 - W_3} \right) \times 100
\]

When \( W_1 \) is the initial n-hexane weight, \( W_2 \) is the weight of n-hexane with scaffold submerged and \( W_3 \) is the weight of remaining n-hexane after removing the scaffold.

3.4. Compressive analysis
To determine the scaffolds’ compressive strength and stress-strain response, the scaffolds underwent compression test. The analysis was done with Universal Testing Machine (Shimadzu EZ-S) with a 500 N load cell and a crosshead speed of 1 mm/min. The scaffolds were compressed until the strain reached 75% to avoid damaging the machinery.

The data obtained was Force (N) versus Stroke (mm). Manual calculations were done to convert force to stress and stroke to strain.

\[
\text{Stress} = \frac{\text{Force}}{\text{Crosssectional area}}
\]

\[
\text{Strain} = \frac{\text{Stroke}}{\text{Specimen's original height}} \times 100
\]

4. Results

4.1. Scaffold fabrication
BG-SF hybrid scaffolds with various ratios of BG:SF were successfully fabricated with gel foaming method. Table 1 shows compositions of scaffold fabricated. (100 uL of Teepol and 200 uL of 5% HF were added to BG100 as surfactant and gelling agent, respectively.)

Table 1. Composition of fabricated BG-SF hybrid scaffold

| Composition | BG (wt%) | SF (wt%) |
|-------------|----------|----------|
| BG100       | 100      | 0        |
| BG90SF10    | 90       | 10       |
| BG80SF20    | 80       | 20       |

4.2. Thermogravimetric analysis
Scaffolds were subjected to TGA (Fig.1). The results were normalized with the amount of moisture trapped inside, and hence started at 150°C onwards. We found that the ash content decreased as the SF content increased. Indeed, the ash contents are as follows: BG100-74.58%, BG90SF10-59.9% and BG80SF20-53.04%. The weight started decreasing steadily at 150°C for all scaffolds. The scaffolds with SF content experienced major weight loss started at 320°C and returned to steady decline at 400°C until 900°C.
4.3. Surface morphology

Scaffolds BG100, 90BG10SF and 80BG20SF were subjected to SEM (Fig.2). Overall, the micrographs showed porous structures throughout the scaffolds (Fig.2C and 2E). We found that the BG100, which only contain bioactive glass, had relatively smooth surface (Fig.2A and 2B). On the contrary, the BG90SF10 and BG80SF20 showed rough surfaces and ball-shaped structure (Fig.2D). The surface of BG80SF20 were covered almost entirely in rough patches (Fig.2E and 2F).
Figure 2. SEM images of scaffolds with different magnifying strength: (A) and (B) are BG100. (C) and (D) are BG90SF10. (E) and (F) are BG80SF20.
4.4. **Porosity**

The scaffold porosity was determined by fluid displacement (Fig. 3). Introduction of SF increased the porosity drastically. The porosity also increased as silk content increases. The increase is in a direct variation trend as the increase from 10%wt SF to 20%wt SF resulted in an increase of 23.88% in porosity.

![Figure 3. Porosity of scaffolds](image)

4.5. **Compression analysis**

Scaffold BG100, BG90SF10 and BG80SF20 underwent compression and the results are shown below (Fig.4). The jagged curves in stress-strain profile were seen in BG100 and BG90SF10 (Fig.4A and 4B). However, BG80SF20 showed no jagged curves previously presented (Fig.4C). Young’s modulus were calculated from stress-strain graph (Table 2). The yield point of BG100, BG90SF10 and BG80SF20 were 8% strain, 11% strain and 22% strain, and with yield strength of 912 kPa, 39 kPa and 29 kPa respectively.

| Scaffold   | Young’s Modulus | Yield strength |
|------------|-----------------|---------------|
| BG100      | 118.34 kPa      | 912 kPa       |
| BG90SF10   | 11.681 kPa      | 39 kPa        |
| BG80SF20   | 1.51 kPa        | 29 kPa        |

![Table 2. Scaffolds’ Young’s modulus and yield strength](image)
Figure 4. Stress-strain responses of scaffolds: (A) BG100. (B) BG90SF10. (C) BG80SF20.

5. Discussion and conclusion

Normally, SF would disintegrate at a temperature over 300° [14]. However, the results suggested that SF and BG underwent some degree of modification and resulted in higher thermal stability. It is also important to note that SF has been functionalized with GPTMS before fabrication. It has been reported that functionalized SF has a slower degradation rate [9], this could carry over to thermal stability. The ash remaining at 900°C decreased when more SF content was added to the scaffolds. This confirms that SF was successfully incorporated to the bioactive glass.

The scaffolds underwent major weight loss during 200°C – 400°C, which is the temperature range where SF disintegrates [14]. The value of these weight loss of each scaffold corresponded with the amount of SF content in the scaffold. BG100, BG90SF10 and BG80SF20 lost 6.02%, 14.44% and 19.41% of its original mass, respectively. This confirms that the more SF added, the more it exists in the scaffolds.

Hybridizing BG with SF yields drastically different mechanical profiles. BG100 exhibit ceramic-like response to compressive load [15]. Ceramics have low compliance but can withstand high compressive force. The stress-strain response produced a steady and rapid climb only to reach the ultimate strength and sudden drastic drop occur as the scaffold shatters. BG80SF20 can withstand less stress than BG90SF10. The changes in the stress-strain graphs’ characteristic can be attributed to the SF content. It was shown that scaffolds with foam structure will slowly fail under crushing load but not entirely due to the failure of struts, causing sudden little drops shown in figure 4B. The overall integrity of the scaffold has not yet failed but some struts have already failed [16]. BG80SF20 (Fig.4C) shows almost linear line of stress-strain response with no sudden drops. This is attributed to crack bridging effect of polymer content covering the surface of the scaffold [17, 18]. Although the ultimate stress was decreased, it should be noted that increasing the SF content can help alleviate the effects of these micro failures of struts. Our results suggest that we may be able to tune the mechanical properties of bioactive
glass to suit different applications by changing the polymeric silk fibroin content. This corresponded with SEM images of the scaffolds. BG90SF10 (Fig.2C) shows very little SF deposit on the surface. In contrast, scaffold BG80SF20 (Fig.2E and 2F) shows abundant deposition of SF content or rough surfaces.

The porosity is also a major factor for compressive strength. The increase of SF content also caused the porosity to increase (Fig.3) and naturally, the structure with higher porosity can withstand less compression. This increase of porosity is an effect from SF being a surfactant [19]. The sol that has more surfactant, when undergoes agitation, will be able to generate and retain more air bubbles resulting in more pores. Also, gelation can occur easier with more SF content.

Drastic change in porosity from BG100 to BG90SF10 and BG80SF20 is likely due to removal of Teepol and HF from the sol. The gelation time and bubble stability due to SF drastically compared to using chemical means.

In conclusion, the BG-SF hybrid scaffolds with varied BG:SF ratios were fabricated. The fabrication is rather straightforward and does not require specialized equipment. The scaffolds were characterized, and this study shows that by increasing SF content we can modify mechanical properties of the scaffolds. The responses of scaffolds to compressive load drastically change when percentage of SF reaches 20% of total scaffold weight. The scaffolds’ compliance increases but at the expense of compressive strength. Scaffolds’ responses to crushing load also change from foam-like response to compliant material’s response possibly due to crack bridging effect of SF content. This could serve as information for future uses regarding micro-failures and scaffold compliance.

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