Abstract

Crystals of hydroxyapatite (HAp) were grown on silk fibers using simulated body fluid (SBF) at a temperature of 37 °C. Two different conditions of silk thread were investigated for efficiency of inducing HAp: i) with sericin protein and ii) without sericin protein. Effect of simulated body fluid (SBF) concentrations at 1.0 × SBF and 1.5 × SBF and sericin protein on HAp growth was discussed. The results showed that HAp was successfully crystallized on silk from both 1.0 × SBF and 1.5 × SBF concentrations. The crystal size obtained from the 1.5 × SBF concentration was larger than that of the 1.0 × SBF concentration. However, it was found that the 1.5 × SBF offered less number of the crystals per unit area than that of the 1.0 × SBF. The physical and chemical compositions of HAp were characterized by XRD, SEM and optical microscopy. This study may be very useful for many applications, especially in biomedical and material science.

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Keywords: Hydroxyapatite; Silk; Sericin; Simulated Body Fluid

1. Introduction

In general, there are many types of a biocompatible material. One of the most outstanding material used is a hydroxyapatite (HAp, Ca_{10}(PO_4)_{6}(OH)_2) due to its superior biocompatibility and chemical composition, which are similar to those of bones and teeth [1]. A variety of porous hydroxyapatite ceramics have been developed for bone replacement allowing cells from the surrounding soft tissues to migrate into the pores to accelerate the proliferation and differentiation of cells [2]. Body fluid and human
blood may be able to fill up into the pores to supply nutrient and mineral ions for growing bone. Therefore, the new bone form and links with the host living tissues. Unfortunately, HAp in the bulk formation faces a big problem on lacking of mechanical properties, e.g. tensile strength and compressive strength [1]. To overcome such limitation of HAp, a composite material based on Hap may be essential. In this report, silk fibers were introduced as the promising material.

Silk fibers mainly comprise fibrous protein, naturally spun into a fiber by a larval from various kinds of insect such as silkworm, scorpion, mite, spider and fly [3]. Silk fibers from Bombyx mori are generally composed of two types of protein assembling similar to a coaxial cable. The inner protein performs as a core filament, called fibroin protein (70 – 80%), whereas the outer coating is sericin protein (20-30%) [2-6]. Silk fibers have been used for long time in textile manufactures; however, instead of being as a cloth product, silk protein, fibroin, also represents potential benefits on medical applications such as drug delivery system, substrate for cell culture, artificial skin and tendon [2]. Fibroin was approved to be a good biocompatible material and perfectly used for medical applications as reported by Altman [5]. However, sericin, another protein coating silk fiber, has shown negative effect on medical application. It was reported that sericin protein could cause adverse problems for biocompatibility and hypersensitivity [5]. Nonetheless, further quantitative investigations may be required.

In this research, the experiment was carried out as in vitro experiment. Simulated body fluid (SBF) was employed as a source of HAp instead of using a human blood. Silk fiber was employed as a seed for inducing HAp crystallization. Two types of silk fiber, with and without sericin protein, were investigated to acquire further information on effect of sericin protein on HAp crystallization. Scanning electron microscope (SEM), optical microscope, and x-ray diffraction (XRD) were used for structural analysis. This novel method may shed light on many important applications in bone replacement implants.

2. Experimental

2.1. Preparation of silk fiber

Raw silk normally covers with sericin protein. To remove sericin protein, raw silk fibers were normally boiled at 100 °C in distilled water for 15 min. The process was repeated five times to ensure that no sericin was left behind. Then, the silk fibers were cut into 5-cm long. To investigate the efficiency of silk fiber on inducing HAp crystallization, the fibers were immersed in the artificial-blood solutions of 1.0 × SBF and 1.5 × SBF concentrations. The samples were kept at 37 °C for 7 days.

2.2. Soaking in simulated body fluid

The simulated body fluid (SBF) is defined as the liquid solution that has the same ion concentrations as those in human blood plasma as reported by Kokubo [7-11, 14]. Thus, in this experiment, the 1.0 × SBF means that the concentration of the prepared solution is equivalent to that of the simulated body fluid (SBF), which has the same ion concentrations as those in human blood plasma. The 1.5 × SBF means that the concentration of the prepared solution is 1.5 times higher than that of the simulated body fluid (SBF) which can be prepared from Table 1. After soaked for 7 days, the samples were removed from the SBF, rinsed deionized water and dried at 50 °C for 2 days.
Table 1. Composition of 1.5 × SBF (pH 7.4) and order of addition of the reagents to de-ionized water at 37°C

<table>
<thead>
<tr>
<th>Order</th>
<th>Reagent</th>
<th>Weight (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NaCl</td>
<td>12.053</td>
</tr>
<tr>
<td>2</td>
<td>NaHCO₃</td>
<td>0.533</td>
</tr>
<tr>
<td>3</td>
<td>KCl</td>
<td>0.338</td>
</tr>
<tr>
<td>4</td>
<td>K₂HPO₄·3H₂O</td>
<td>0.347</td>
</tr>
<tr>
<td>5</td>
<td>MgCl₂·6H₂O</td>
<td>0.467</td>
</tr>
<tr>
<td>6</td>
<td>1M HCl</td>
<td>50 ml</td>
</tr>
<tr>
<td>7</td>
<td>CaCl₂</td>
<td>0.438</td>
</tr>
<tr>
<td>8</td>
<td>Na₂SO₄</td>
<td>0.108</td>
</tr>
<tr>
<td>9</td>
<td>C₄H₁₁NO₃ (Tris)</td>
<td>9.177</td>
</tr>
<tr>
<td>10</td>
<td>1M HCl</td>
<td>8.5 ml</td>
</tr>
</tbody>
</table>

The 1.0 × SBF concentrations was prepared by a dilution of the 1.5 × SBF concentration as the following equation:

\[ C_1 V_1 = C_2 V_2 \quad (I) \]

where \( C_1 = 1.5 \), \( V_1 = \) volume of 1.5 × SBF
\( C_2 = 1.0 \), \( V_2 = \) volume of 1.0 × SBF

2.3. Characterization

Morphological structure of silk fibers (before and after soaking in SBF) was characterized by optical microscope (OLYMPUS BX 60, Japan) using inverted light source and SEM (JEOL-JSM 6510) at 10 kV.

Silk structure was analyzed by x-ray diffractometer (XRD, Rigaku RINT 2000) with CuKα radiation (\( \lambda = 1.54059 \) Å). To do XRD characterization, the HAp film was prepared via drop-dried method on silicon wafer (100) as shown in Fig. 1.

![Schematic diagram of sample preparation x-ray diffraction (XRD)](image)
Fig. 1 shows schematic diagram of sample preparation for XRD investigation. i) 0.2 g of dried silk from 7 days of SBF incubation was immersed in 10 ml of deionized water. ii) The sample was sonicated in an ultrasonic bath for 30 min, then, the silks were removed from dispersion. iii) The dispersion was centrifuged at 14,000 rpm for 1 hr, and supernatant was then removed. iv) 50 – 60 μl of the sediment was dropped on silicon surface and kept at 70 °C in the oven for 20 min. v) the drop deposition process was repeated for 3 times and then the sample was ready for XRD characterization.

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3. Results and discussion

Fig. 2(a) and (b) show optical images of silk fibers before soaking in SBF. The diameter of the fiber is about 8-10 μm. And, clearly, there is no any big particle attached on their surface. After soaking in the 1.0 x and 1.5 x SBF concentrations for 7 days, the fibers were covered with HAp crystals as shown in Fig. 2(c)-(f). The HAp crystals were clearly observed on the SEM images as shown in Fig. 3(a)-(f).

![Fig. 2](image-url)
Silk fibers of without sericin protein offered less number of HAp crystal per unit area than that of ones with sericin. The difference in the number of the crystal per unit area may be resulted from the sericin protein. The protein may act as a sponge lowering nucleation barrier of HAp crystallization when the adsorbed molecules have enough activation energy to overcome the barrier, the crystal simultaneously forms. Moreover, the size of crystals from different SBF concentration was investigated. It was found that the $1.5 \times SBF$ concentration offered a larger size of crystal as shown Fig. 2(d) and (f) and Fig. 3(e) and (f). However, most of the crystal found in $1.5 \times SBF$ concentration resemble to another phase of calcium phosphate, see Fig. 3(f). This may be due to the high concentration of SBF that enables a rapid growth and the mismatch of Ca:P ratio which is not 1.67 [13]. To have better understanding on this phenomenon, further quantitative measurement may be required.

Fig. 3. SEM images of HAp on silk fibers (a) without sericin in $1.0 \times SBF$; (b) without sericin in $1.5 \times SBF$; (c) and (e) with sericin in $1.0 \times SBF$; (d) and (f) with sericin in $1.5 \times SBF$
Fig. 4 shows XRD pattern of HAp from drop-dried method of 1.0 × SBF. The XRD peaks were marked at the same position of HAp (Calcium Phosphate Hydroxide; JCPDS No. 76-0694) [1, 11]. This result confirmed that HAp crystals were successfully grown on silks surface.

4. Conclusions

Hydroxyapatite (HAp) was successfully grown on silk fiber using simulated body fluid (SBF) at a temperature of 37 °C. Sericin protein has shown a significant impact on growing HAp by reducing nucleation barrier. Different concentrations of SBF offer different results on HAp crystallization. 1.0 × SBF offers many crystals of HAp, whereas 1.5 × SBF gives large crystals, which might be the other phases of calcium phosphate. This result may contribute to some useful information, essential for many applications in biomedical and material science.

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References


