Growth of hydroxyapatite on sericin-coated silk fibers using simulated body fluid at different periods of soaking time

Onanong Sukjai[1,2*], Piyapong Asanithi[1*], Pichet Limsuwan[1,2] and Supanee Limsuwan[1]

[1] *Applied Nanotechnology Laboratory (ANT Lab), Department of Physics, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand*
[2] *Thailand Centre of Excellence in Physics, Commission on Higher Education, Ministry of Education, Bangkok 10400, Thailand*
** Corresponding authors: Tel. +66 83 2316089, +66 2 4708868, Fax. +66 2 8725254*

E-Mail: onanongsukjai@gmail.com, asanithi@hotmail.com

Abstract
Hydroxyapatite (HAp) was grown on sericin-coated silk fibers using simulated body fluid (SBF) with a concentration of 1.5×standard SBF at 37 °C, for two periods of soaking time, including 7 days and 30 days. The results from both soaking time conditions showed that, HAp is able to grow on sericin-coated silk fibers. Also, it was found that soaking time has played an important role on phase of HAp. For 7 days, there was only HAp crystal grown on sericin coated silk fibers. But, for 30 days, there was HAp and calcium phosphate crystals grown on sericin-coated silk fibers. This indicates that the optimum day for growth of HAp crystal is 7 days. Moreover, the particle size of HAp crystal grown at 7 days is smaller than that of 30 days.
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Keyword: Hydroxyapatite, Simulated body fluid, Sericin, Silk fiber

1. Introduction

Human bone is composite material. About 70 wt% is mineral phase. It is hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) with lower crystallinity. About 30 wt% is organic matrix which is mainly composed of Type I collagen [1-4]. Hydroxyapatite (HAp) is an excellent material for application in biocompatibility, osteo-conductivity and bioactivity [5-7]. It has been studied and developed for bone replacement and remodelling process of cells surrounding soft tissues at the bone-implant interface because of the special characteristics of hydroxyapatite. Moreover, HAp is a perfect host for living tissues since it allows human blood to diffuse into its porous structure to supply nutrient and mineral ions for accelerating the proliferation and differentiation of bone cells [8].

Silk fiber is naturally originated from silkworm (*Bombyx mori*). It consists of protein-based fiber with two types of protein; sericin and fibroin. Fibroin is the core filament and benefit for medical applications, substrate for cell culture, artificial skin and tendon [1]. It was approved for its biocompatibility which can be perfectly used in medical application as reported by Altman [9]. The outside coated is sericin which is a water soluble protein and apply to use in biological and medical materials due to its antibacterial, UV resistant property [10], lipid oxidation [11] and anti-tumour property in immunogenicity [12-13].

In this research, sericin-coated silk fiber was used as a seed to induce HAp crystal by soaking in SBF of different periods of soaking times. HAp crystals on the silk surface were washed and dried for morphological investigation using field-emission electron microscopy (FE-SEM). The structure of HAp crystals was characterized by X-ray diffraction (XRD).

2. Experimental

2.1. Silk fibers preparation

Sericin-coated silk fibers were prepared by rinsing the raw silk fibers with DI water for three times to remove dust or any impurity and dried in an oven at 37 °C for 1 day. Then, silk fibers were cut into 5 cm long for soaking in the SBF solution.

2.2. Simulated body fluid preparation

Simulated body fluid (SBF) is a liquid solution that contains the same ion concentrations as human blood plasma as reported by Kokubo [8,14-19]. Also, the 1.5×standard SBF concentration used in this work is followed the Kokubo's recipe as shown in Table 1. In this work, the SBF with the concentration of 1.5 times of human blood was used as a stock solution.

Table 1 Reagent addition order in DI water for 1 litre of SBF

Order	Reagent	Source of reagent	Amount
1	NaCl	Ajax Finechem	12.053 [g]
2	$NaHCO_3$	Ajax Finechem	0.533 [g]
3	KCl	Ajax Finechem	0.338 [g]
4	$K_2HPO_4 \cdot 3H_2O$	Carlo Erba Reagent	0.347 [g]
5	$MgCl_2 \cdot 6H_2O$	Ajax Finechem	0.467 [g]
6	1M HCl	J.T. Baker	50 [ml]
7	$CaCl_2$	Ajax Finechem	0.438 [g]
8	Na_2SO_4	Ajax Finechem	0.108 [g]
9	$C_4H_{11}NO_3$ (Tris)	Ajax Finechem	9.177 [g]
10	1M HCl	J.T. Baker	8.5 [ml]

2.3. Growth of HAp on silk fibers

The prepared SBF was filled into two plastic bottles with 30 ml. 0.2 g of silk fibers which are already prepared and cut into 5 cm long was put in each plastic bottle. The samples were divided into two groups for 7 days and 30 days.

All two samples were kept in an incubator at 37°C. The first group has been soaked for 7 days. The second group was soaked for 30 days and change SBF every 7 days to constant ion concentration in SBF. After due to soak time, the samples were washed with DI water and dried in an air oven at 37°C for 1 day.

All two dried silk fibers with HAp crystals on the surface were used for investigating morphology by thermal SEM and FE-SEM. Subsequently, HAp crystals on the silk fibers were removed by sonicating method. 0.2 g of each soaked silk fibers were put in 10 ml of DI water and sonicated for 30 min. Then, sediment of HAp was drop on silicon wafer (100) and dried at 70 °C for 20 min and repeat drop-dried method for 3 times to increase thickness of HAp film. The dried HAp samples were used for XRD characterization.

2.4. Characterization

The structure of HAp crystals was characterized by XRD (Rigaku, RING 2000) with CuK_α in the 2θ range of 20°-55° with a scan step of 0.02°. The morphological structure of HAp crystals on silk fibers was characterized with two methods. First was thermal SEM (JSM-JEOL, 5800LV) at 10 kV and 10 nm gold coating. Second was FE-SEM (JSM-JEOL, 6301F) at 5 kV and 30 nm gold coating.

3. Result and discussion

Figure 1 shows XRD patterns of the crystals on silk fibers after soaked in SBF. Figure 1 (A) is XRD pattern of the crystals on silk fibers 7 days soaked in SBF. All peaks show hexagonal phase of HAp ($Ca_{10}(PO_4)_6(OH)_2$). It is the same phase as in human bone. It could be indexed based on JCPDS No. 09-0432.

Figure 1 (B) is the crystals on silk fibers 30 days soaked in SBF's XRD pattern. The peaks show HAp ($Ca_{10}(PO_4)_6(OH)_2$) and calcium phosphate ($Ca_3(P_5O_{14})_2$) based on JCPDS No. 09-0432 and No. 53-1538. Here long period of soaking time effects the formation of HAp crystals. Sericin has OH group, COOH group and NH group. These are main groups to coordinate with Ca^{2+} in the SBF. When it takes long period of soaking in SBF, most of these groups were boned or linked with Ca^{2+}. So, the solution was preferred to form in calcium phosphate next [20]. Furthermore, Ca/P ratio should be 1.67 to form HAp. SBF was changed every 7 days for 30 days. This effects to Ca/P ratio. So, the crystal on silk fibers form in another phase of calcium phosphate not HAp [21].

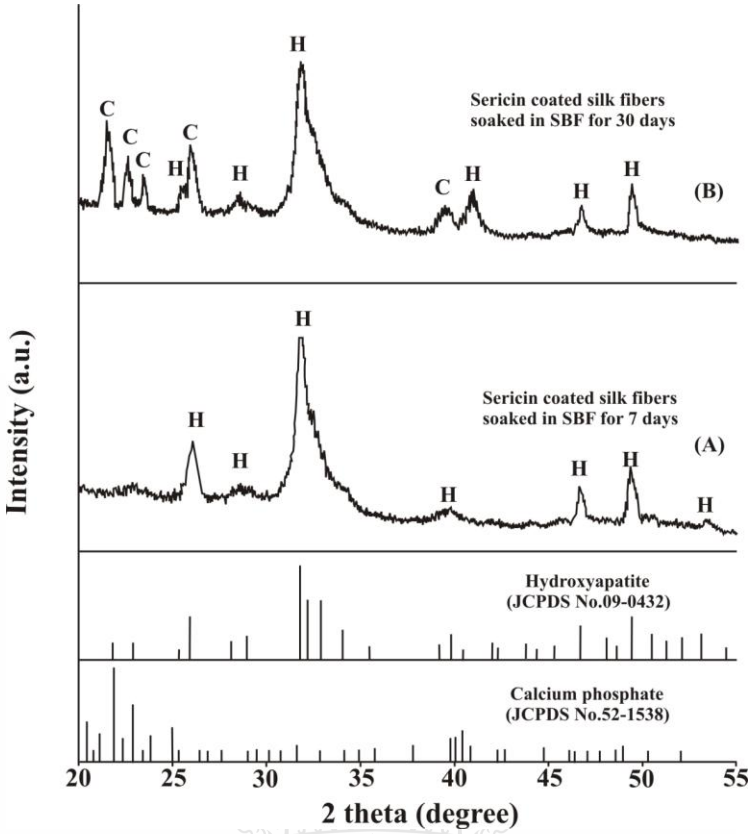

Figure 1 XRD patterns of HAp and calcium phosphate crystals on silk fibers after soaked in SBF at 7 days and 30 days

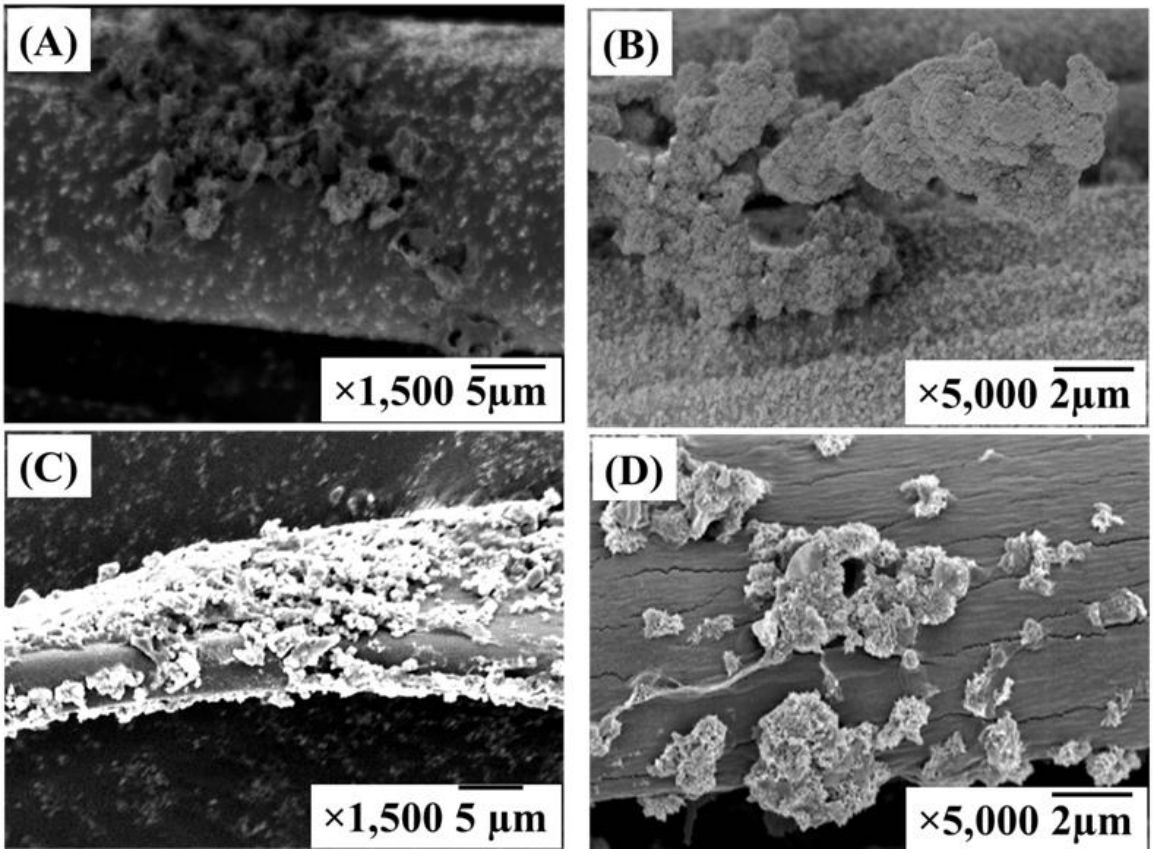

Figure 2 SEM images of calcium phosphate and hydroxyapatite crystals on various soaked time (A, B) silk fibers soaked in SBF for 7 days and (C, D) silk fibers soaked in SBF for 30 days

Figure 2 (A) shows SEM image of HAp crystals at a magnification of 1,500 on silk fibers after soaked in SBF for 7 days. The HAp crystal size is about 2.5-7 μm. After characterized with FE-SEM, HAp crystal was seen clearly. Figure 2 (B) shows FE-SEM image of HAp crystal at a magnification of 5,000 on silk fiber after soaked in SBF for 7 days. It shows that big HAp crystal is composed of small crystals and looks like a cauliflower. The small crystals size is about 0.1-0.25 μm. Figure 2 (C) shows SEM image of HAp at a magnification of 1,500 on silk fibers after soaked in SBF for 30 days. The size is about 1.5-5 μm. Figure 2 (D) shows FE-SEM image of HAp at a magnification of 5,000

for 30 days SBF soaked. There are HAp crystals and calcium phosphate crystals on silk fibers. These results were agreed with the XRD pattern in Figure 1. The results in figure 2 shows that increased soaking time increase crystal size. Because long soaking time, molecule of calcium in SBF can grow to big crystal than shot time. When change SBF every 7 days, it increases ion concentration to form the crystal in an island shape. 30 days of soaking time might be too long. Soaked time at 14 days or 21 days will be better but they have to be studied in the future.

4. Conclusions

Hexagonal phase of hydroxyapatite (HAp) was successfully induced on sericin-coated silk fibers. The crystal size is about 0.1-0.25 μm and is grown in an island shape. After increasing soaking time, the crystals size was increased and another phase of calcium phosphate crystals was formed. The size of HAp crystal can be controlled by soaking time which is the method to select the optimum condition for growing HAp crystals.

Acknowledgments

This research had partially been supported by Thailand Center of Excellent in Physics (ThEP) and King Mongkut's University of Technology Thonburi under The National Research University Project

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