Inorganic/Organic Coating Layer to Induce Apatite Formation in DPBS

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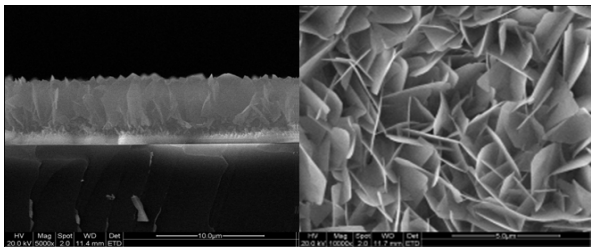
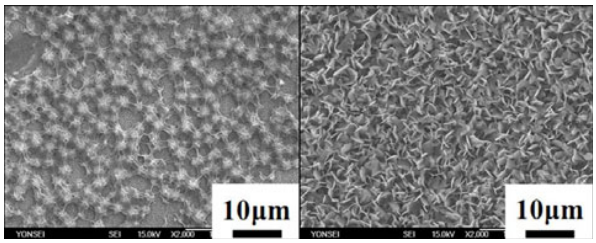
Statement of Purpose: Although there are still some controversies, apatite forming ability of implants in simulated body fluid (SBF) is an object of much interest as it could give some indication of *in vivo* behaviors [1]. In the present study, calcium phosphate thin film as an inorganic coating layer and silk fibroin (silk II structure, water-insoluable) as an organic coating layer was formed on commercially pure (CP) titanium, respectively. Samples were then incubated in Dulbecco's Phosphate buffer saline containing calcium chloride (DPBS) to evaluate apatite forming ability and to investigate the detailed insight of apatite nucleation and growth.

Methods: Commercially pure titanium discs (grade IV, 10 mm in diameter and 2 mm in thickness) were used as substrates. After sonicating in acetone and then deionized water, the samples were dried in a nitrogen stream prior to the deposition process. Calcium phosphate film was synthesized by electron beam evaporation [2]. For silk fibroin coating, degummed fibroin fiber was first dissolved in boiling calcium chloride aqueous solution (42%) for 10 min. The solution was dialyzed against deionized water for 3 days at 4 ℃ in order to remove excess calcium chloride. After being condensed, the fibroin solution was centrifuged at 10,000 rpm for 10 min, filtered, and then stored at 4 ℃. The fibroin solution was then diluted to 3% for use. After the substrates were soaked in 5 M NaOH solution for 24 h at 80℃, they were cleaned in distilled water and then dried. 30μL fibroin solution was dropped to the upper surface of Ti disk. After dried, 80% (v/v) ethanol was used to induce the structural transition from unstable silk I to water-insoluble silk II. Calcium phosphate and silk fibroin coated samples were incubated in Dulbecco's Phosphate buffer saline containing calcium chloride (DPBS).). The surfaces of samples were examined with field emission scanning electron microscopy (FESEM), X-ray diffraction and X-ray photoelectron spectroscopy.

Results: Figure 1(a) shows scanning electron micrographs of calcium phosphate coated sample incubated in DPBS at 37 °C for 24 h (left: cross section, right: top). Newly formed apatite appeared on coated surface even after immersion in DPBS for 15 min. The apatite crystals grew from small and thin curved units to straight and flake-like units for the prolonged incubation. XRD pattern shows that the new peaks of bone-like apatite (near 2θ=26° and 2θ=32°) began to appear after 1 h immersion and the intensity increased with the incubation time, which demonstrated that more apatite was formed. Although silk fibroin coated sample showed slower apatite formation as shown in Figure 1(b) the amount of apatite was also increased with immersion time demonstrating positive potential for improving implants fixation and spontaneous binding to neighboring bone. Currently, the effect of incorporating protein and post treatments of silk fibroin coating on apatite formation are currently investigating.



(a)



(b)

Figure 1. SEM micrographs of (a) calcium phosphate coated sample incubated in DPBS for 24 h, (b) silk coated sample incubated for 6 hr (left) and 24 h (right).

Conclusions: Apatite began to nucleate on e-beam deposited calcium phosphate film even after 15 min immersion in DPBS. Silk fibroin coating exhibited apatite forming ability. Structural stability and controlling in biodegradation of silk fibroin coating have wide interest for the potential biomedical applications

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References:

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