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Preparation and characterization of modified polyvinylalcohol-hydroxyapatite composites

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Polymer-hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) composite is a promising candidate for hard tissue engineering scaffold materials, particularly bone and teeth. The form of the composite depends on the shape of the base-polymer film. We demonstrated that the shape of the base polymer film was controlled by photo-pattering technique with a photo-crosslinkable polymer and hybridization of the shaped polymer film and HAp was performed by alternative soaking process. Poly(vinyl alcohol) with *trans*-cinnamate moiety as chromophoric groups (P(VA-VCI)) was spin-coated on various substrates, glass, metals such as titanium, aluminum, stainless steel, and used for photo-patterned film formation by UV-light irradiation. The patterned P(VA-VCI) film on the substrate was processed alternate soaking process for preparation of P(VA-VCI)-HAp composites. We confirmed that formation of HAp in the P(VA-VCI) matrix by Fourier transform infrared spectroscopy and X-ray diffraction technique. Microscopic observations of the photo-patterned P(VA-VCI)-HAp composites were also performed. The border line between the composite and the substrate was clearly observed even after the alternative soaking process.
Key words: Photo-crosslinkable polymer, Hydroxyapatite, Scaffold, Alternative soaking process

1. INTRODUCTION

Hydroxyapatite (HAp) is an attractive biomaterial because of its high bioaffinity. HAp is part of the inorganic constituent of natural bone and teeth. Therefore, HAp coating has been widely used in orthopedics and dentistry to improve the tissue compatibility of various materials. Polymer-HAp composites have been prepared and used for scaffold materials of hard tissue, such as bone and teeth. Polymers for the composites are chitosan [1-3], polyetheretherketone [4], polyacrylamide gel [5], polyhydroxyalkanoate [6], poly(lactide-*co*-glycolide) [7, 8], poly-*L*-lactide [9], hydrophilic polymer-grafted poly(ethylene) [10], and poly(α-hydroxy ester) [11].

Polymer-HAp composites have been prepared by various methods. For example, Kokubo *et al.* developed a biomimietic process and found out that an HAp layer could be formed on polymer films [12, 13]. Akashi *et al.* prepared polymer-HAp composites by alternative soaking process [10]. They have been studying the biological functions the composite materials for development of the hard tissue engineering scaffold. The materials' shape and size depend on the shape and size of the base polymer film. Thus control of the film shape and size will bring us flexible design of the hard tissue engineering scaffold. Yao *et al.*, reported micro pattern formation of ceramic thin film by synthesis from aqueous solution using resist pattern as a mold [14]. They prepared HAp films by biomimetic method [12, 13, 15-17]. This process is as follow; first, a substrate was set in contact with granular particles of bioactive CaO SiO_2 based glass in SBF (Na^+ 142.0, K^+ 7.5, Ca^{2+} 2.5, Mg^{2+} 1.5, HCO^{3-} 4.2, Cl^- 148.0, HPO_4^{2-} 1.0 and SO_4^{2-} 0.5 mmol dm^{-3}), then a number of apatite nuclei were formed on the substrate. Second, the substrate was soaked in 1.5SBF with ion concentrations 1.5 times those of SBF at 36.5° C, and the apatite nuclei grew in situ. The growth rate of the HAp layer was very slow. They needed 4 day period for the first step and 4 days period for the second step. The apatite layer was formed on the substrate by using photoresist polymer as a mold which should be removed after the process.

In this paper, we demonstrated preparation of photo-patterned polymer-HAp composites by using photo-patterning technique and successive alternative soaking process. Fig. 1 shows the schematic presentation of the preparation procedure of a photo-patterned polymer-HAp composite.

The first step in Fig. 1 is photo-patterning process of polymer film on a plate. The second step is development of the film and creation of the photo-patterned polymer film. The third step is successive alternative soaking process to deposit HAp in the film. We needed only 2 day period for the preparation method of the photo-patterned polymer-HAp composite.

2. EXPERIMENTAL

2.1. Materials

Poly(vinyl alcohol)(PVA) was purchased from Ishizu Co. Its average degree of polymerization was 500. *trans*-Cinnamyl chloride was prepared from *trans*-Cinnamic acid (Tokyo Kasei Co.) and thionyl chloride(Ishizu) [18]. The *trans*-cinnamyl chloride was purified with distillation under reduced pressure(main fraction 110-111° C, 5 mmHg).

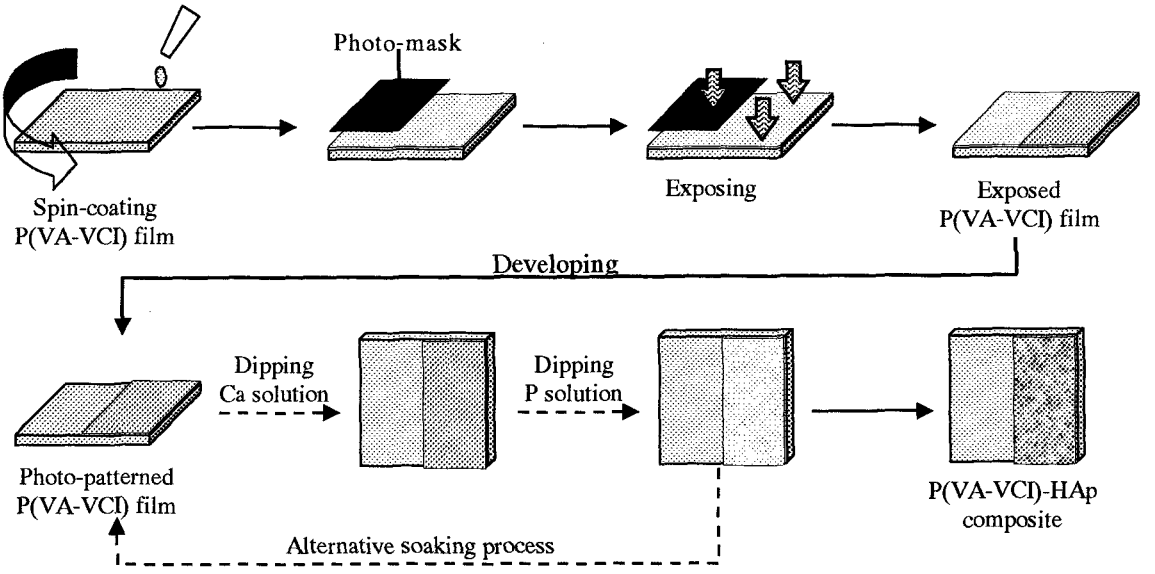

Fig. 1 Preparation procedure of photo-patterned P(VA-VCI)-HAp composite.

2.2. Preparation of poly(vinyl alcohol-*co*-vinyl *trans*-cinnamate)

Poly(vinyl alcohol-*co*-vinyl *trans*-cinnamate), P(VA-VCI) (Fig. 2) was prepared PVA and *trans*-cinnamyl chloride by interfacial synthesis technique [19]. Typical procedure is as follows.

PVA(1.11 g) was dissolved into 25 ml of water. Sodium hydroxide solution which is sodium hydroxide (4.0 g) dissolved into 25 ml water was slowly added to the aqueous PVA solution, and then 25ml of 2-butanone was also added to the solution (Solution A).

Following procedure performed in dark room. *trans*-Cinnamyl chloride (5.02g) was dissolved into the mixed solvent (27 ml of 2-butanone and 6 ml of toluene), (Solution B). The solutions A and B were cooled at 0 °C. Then the solution B was added to the solution A. The mixed solution was kept at 0±2 °C and stirred 300 rpm for 90 min. Then the mixed solution left at rest and the upper layer solution (organic layer) was collected. The organic layer solution was poured into 60 ml of methanol and the participated polymer was filtered off. The resulted polymer was dissolved into 2-butanone and re- precipitated into water. The purified polymer was collected and dried at 70 °C for 24 h. A content of trans-cinnamate units in the polymer was estimated from ^{1}H-NMR spectrum of the polymer. This study used that the content of *trans*-cinnamate moiety was 79.4 mol% in the P(VA-VCI).

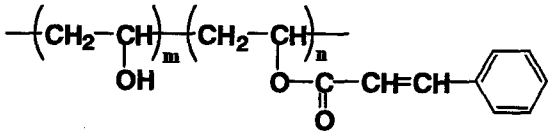

Fig. 2 Structure of P(VA-VCI). The content of *trans*-cinnamate moiety was 79.4 mol%.

2.3. Spin-coated of P(VA-VCI) film on substrate and photo-pattering on the film

Following procedure performed in dark room. Purified P(VA-VCI) (0.148 g) dissolved into 14.0 ml of 2-butanone. A P(VA-VCI) film on a substrate (25×25 mm), such as glass, aluminum, stainless steel and titanium, was prepared by spin-coated technique at 1500 rpm for 3 s. After the solvent of 2-butanone evaporated, photo-mask was placed on the P(VA-VCI) film and irradiate for pattering on the film. Most irradiations were conducted at room temperature using a UV-lamp (SPECTROLINE ENF-240C/J, SPECTRONIC Co.) for 30 min. The exposed film was dipped into the development solvent is the mixture solvent (toluene, xylene, and 2-methoxyethyl acetate, 1:1:1, by vol.) for 30 s.

2.4. Hydroxyapatite deposition in the photo-patterned P(VA-VCI) films

Hydroxyapatite deposition in the photo-patterned P(VA-VCI) film was performed with alternate soaking process [10]. The first, a photo-patterned film with a substrate, such as glass, metal, was soaked in 40 ml of Ca solution ($CaCl_2$/tris(hydroxymethyl)aminomethane-HCl solution; pH 7.40, $[Ca^{2+}]$=0.20 mol/l) at 37.5° C for 2 h. The film was removed from the Ca solution, and was rinsed with excess distilled water to remove the attached excess Ca solution. The film was soaked in 40 ml of P solution (Na_2HPO_4; pH 9.11, $[PO_4^{3-}]$=0.12mol/l) at 37.5° C for 2 h. The film was removed from the P solution, and was rinsed with excess water. We controlled the amount of the deposited hydroxyapatite in the film by number of the above procedure repeating times.

3. RESULTS AND DISCUSSION

3.1. Photo-patterning of (P(VA-VCI)) film

The microscopic image of the photo-patterned P(VA-VCI) film on the glass substrate shows Fig. 3 (a). The film was homogeneous and thickness was 6 ㎛.

The boundary line between the substrate and the photo-patterned P(VA-CVI) film was observed very clear. We also prepared P(VA-VCI) film on various substrate plates was titanium, stainless steel and aluminum. The films were homogeneous and thickness was 7–9 μm. The boundary line between the substrate and the photo-patterned P(VA-CVI) film on the metal plate was also observed very clear.

3.2. Alternative soaked photo-patterned P(VA-VCI) films

The microscopic images of alternative soaked photo-patterned P(VA-VCI) films are shown in Fig. 3 (b)-(d). The film became clouded homogeneously with increase in the number of soaking process cycle. The magnified surface images of P(VA-VCI) film before alternative soaking is shown in Fig. 3(e) and after 15th cycle alternative soaking is shown in Fig. 3(f). Many small particles were observed in Fig. 3(f).

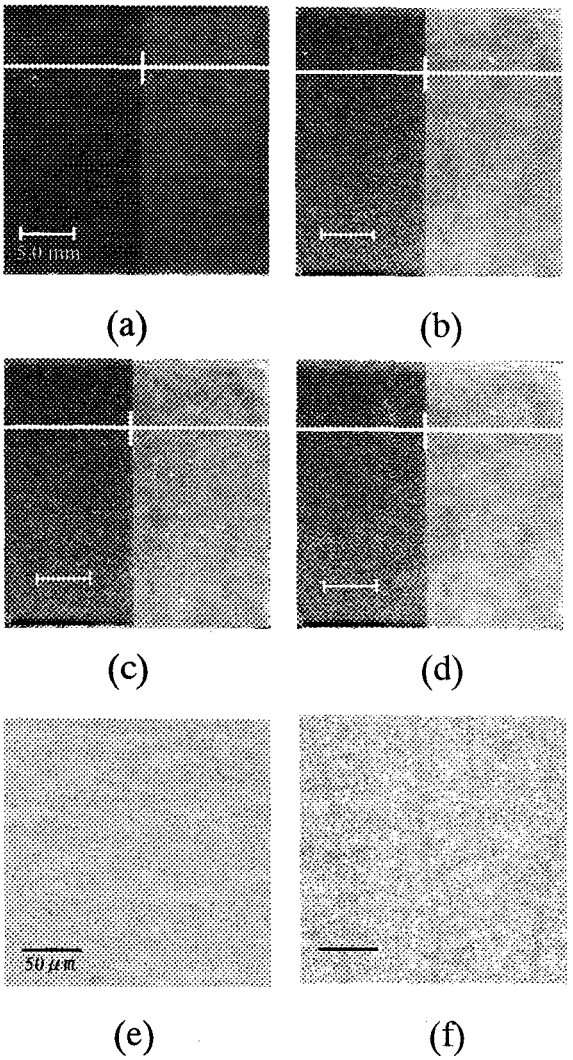

Fig. 3 Microscopic images of photo-patterned P(VA-VCI) films before and after alternative soaking process, before soaking(a), 5(b), 10(c), and 15cycles (d). Left side from the center line was the masked area and right side was the exposed area. Scaling bar is 5 mm. Surface images of the films before and after alternative soaking process, before soaking (e) and after 15 cycles (f). Scaling bar is 50 μm.

The film thickness and weight also increased with the succession of the process. Fig. 4 shows relationship between the number of soaking process cycle and the thickness of the patterned P(VA-VCI) film on the glass substrate. Increase in the cycle number increased the film thickness. Liner fitting of the plots suggests that the increase in thickness is 0.34 μm per soaking process cycle. The result also indicates that increase of the film thickness is uniformly.

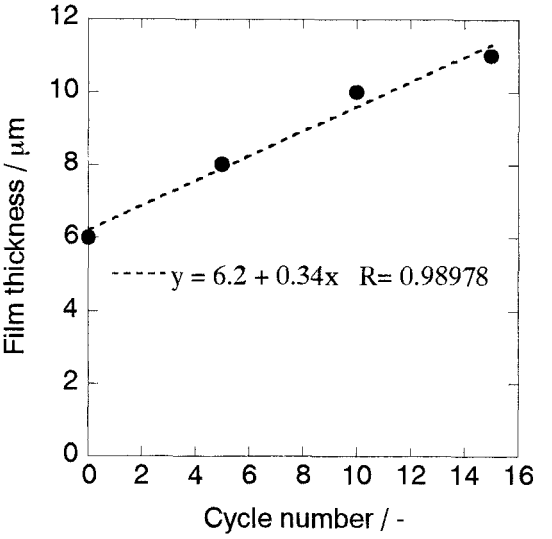

Fig. 4 Relationship between alternative soaking cycle number and P(VA-VCI) film thickness.

Fig. 5 shows X-ray diffraction patterns of alternative soaked photo-patterned P(VA-VCI) films. The X-ray diffraction pattern of hydroxyapatite in the JCPDS Card is also shown Fig. 5 (f). Two large peaks at 25.8 ° and 32.8 ° are characteristic peaks for HAp. The large two peaks were observed in the patterns in Fig.5 (b)–(d). The result suggests that the white particles in the P(VA-CVI) films are HAp. The peaks in the XRD charts were broad.

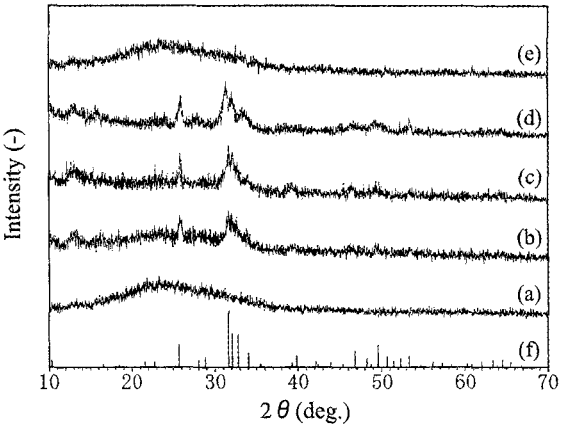

Fig. 5 X-ray diffraction patterns of P(VA-VCI) films before and after alternative soaking process, before soaking (a), 5 (b), 10 (c) and 15 cycles (d). The pattern in Fig. 5 (e) is non-exposed part of the plate (the left side area of Fig. 3 (d)). Fig. 5 (f) shows the peak positions in the JCPDS-card of hydroxyapatite.

This indicates that the deposited hydroxyapatite in the film is partially amorphous. Fig. 5(e) is the XRD pattern of the masked area (non-exposed area, the left side from the center line) on the plate shown in Fig. 3 (d). The peaks in Fig. 5(e) were broad. This indicates that deposition of HAp did not occur without P(VA-CVI) film in the substrate.

Fig. 6 shows FTIR spectra of the patterned P(VA-CVI) film and the film treated with the alternative soaking process. The overlapped peaks located around 1000 cm^{-1} are originated by phosphate modes (Fig. 6 (b) and (c)). The split bands, mainly at 1030 and 1090 cm^{-1}, suggests the formation of HAp (Fig. 6(b)). In Fig. 6(c) carbonate bands were observed at 879, 1415 and 1455 cm^{-1}. Thus, calcium carbonate co-deposited in the film. Molecular and adsorbed water bands at 1640 and 3400 cm^{-1} are also observed in both spectra in Fig. 6(b) and (c).

We conclude that deposition mechanism of HAp in the P(VA-VCI) is as follow. A photo-patterned P(VA-VCI) film with a substrate such as glass, metal, was soaked in the Ca solution at 37.5° C for 2 h. Some amounts of calcium ions are trapped in the polymer film. Then film was soaked in P solution at 37.5° C for 2 h. Some amounts of phosphate ions also trapped in the film containing calcium ions. Formation of HAp occurs in the polymer film. Rinse process between Ca solution soaking and P solution soaking prevents direct formation of HAp on the glass substrate without P(VA-VCI) film.

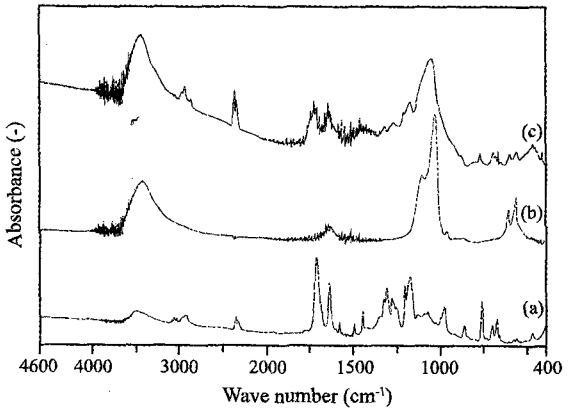

Fig. 6 Infrared spectra of P(VA-VCI) film. Before alternative soaked P(VA-VCI) film (a), the surface (b) and the inside (c) of alternative soaked P(VA-VCI) films after 15 cycles.

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