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Preparation of photo-patterned polymer–hydroxyapatite composites

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Abstract

A polymer–hydroxyapatite (HAp) composite is useful as hard tissue engineering scaffold materials for bone and teeth, particularly. It is necessary to control the form of the composites precisely, because form of bone and teeth is unique and specific. We demonstrated that the shape of a base polymer film was controlled by a photo-pattering technique. Hybridization of the photo-patterned polymer film and HAp was performed by alternative soaking of the film into calcium chloride aqueous solution and phosphate buffer solution. Poly(vinyl alcohol) bearing *trans*-cinnamate moieties as chromophoric groups (P(VA-VCI)) was spin-coated on various substrates, glass, metals such as titanium, aluminum, stainless steel. The spin-coated film was covered with a patterned photo-mask, exposed by using UV lamp and finally developed by dipping into mixed organic solvent. The patterned P(VA-VCI) film was processed by the alternate soaking process for preparation of P(VA-VCI)–HAp composites. We confirmed that formation of HAp in the P(VA-VCI) film by 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 polymer–HAp composite film and the substrate is clearly observed even after the alternative soaking process. This method is a method for being very useful for producing the polymer–HAp composite with the form of thought way.

Keywords: Photo-crosslinkable polymer; Hydroxyapatite; Scaffold material

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1. Introduction

New-type materials which consist with organic and inorganic compounds have been prepared in last decade [1]. The materials have various functions and specific nature because of their improved mechanical, thermal, optical, or chemical properties relative to the organic or inorganic materials alone. Polymer–ceramic particle hybrid materials have been also investigated because of their wide application, pervaporation membranes [2,3] and ion conducting materials for batteries [4–6]. Hydroxyapatite ($Ca_{10}(PO)_6(OH)_2$, HAp) is one of the ceramic materials with bioaffinity. HAp is part of the inorganic constituent of natural bone and teeth. Polymer–HAp composites have been prepared by various methods. For example, Kokubo et al. [7,8] developed a biomimietic process and found out that an HAp layer could be formed on polymer films. Akashi et al. [9] prepared polymer–HAp composites by alternative soaking process. 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. [10] reported micro pattern formation of ceramic thin film by synthesis from aqueous solution using resist pattern as a mold. They prepared HAp films by biomimetic method [7,8, 11–13].

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 days 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.

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In this paper, we demonstrated preparation of photo-patterned polymer–hydroxyapatite (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.

Step 1 in Fig. 1 is photo-patterning process of polymer film on a plate. Step 2 is development of the film and creation of the photo-patterned polymer film. Step 3 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) and thionyl chloride (Ishizu) [14]. The *trans*-cinnamyl chloride was purified with distillation under reduced pressure (main fraction 110–111 °C, 5 mmHg).

2.2. Preparation of poly(vinylalcohol-co-vinyl trans-cinnamate) (P(VA-VCI))

Poly(vinylalcohol-*co*-vinyl *trans*-cinnamate) (P(VA-VCI)) was prepared from PVA and *trans*-cinnamyl chloride by interfacial synthesis technique [15]. Typical procedure is as follows

PVA (1.11 g) was dissolved into 25 ml of water. Sodium hydroxide solution (4.0 g/25 ml) was slowly added to the aqueous PVA solution and then 25 ml of 2-butanone was also added to the solution (PVA solution). *trans*-Cinnamyl chloride (5.02 g, 3.01×10^{-2} mol) was dissolved into the mixed solvent (27 ml of 2-butanone and 6 ml of toluene), (CI solution). PVA solution and CI solution were cooled at 0 °C. Then the CI solution was added to the PVA solution (stirring at 300 rpm) at the same time in the dark. The temperature of the solution was kept at 0 ± 2°C for 90 min. Then the solution left at rest and the upper layer solution (organic layer) was collected. The organic layer solution was poured into methanol (60 ml) and the participated polymer was filtered off. The resulted polymer was dissolved into 2-butanone and re-participated into water. The purified polymer was collected and dried at 70 °C for 24 h. (Yield 1.1 g.)

A content of *trans*-cinnamate units in the polymer was estimated from ^{1}H-NMR spectrum of the polymer. Unless otherwise mentioned, the content of *trans*-cinnamate moiety in the P(VA-VCI) used in this study was 79.4 mol%.

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

A solution of P(VA-VCI) for spin-coating process was prepared from 0.148 g of P(VA-VCI) and 14 ml of 2-butanone and the solution was stored in the dark. A P(VA-VCI) film on a plate (25 mm × 25 mm), such as glass, titanium, stainless steel, aluminum, was prepared by spin-coating technique at 1500 rpm for 3 s. The thickness of the resulted film was about 6 μm. A mask plate was placed on the P(VA-VCI) film and irradiated 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. Development of the exposed film was performed by dipping the plate into the development solution (toluene, xylene, and 2-methoxyethyl acetate (1:1:1, by vol.) for 30 s.

2.4. Hydroxyapatite deposition in the photo-patterned films

The apatite deposition in the photo-patterned film was performed with modified alternate soaking process reported by Akashi et al. [9]. The typical process was performed as follows

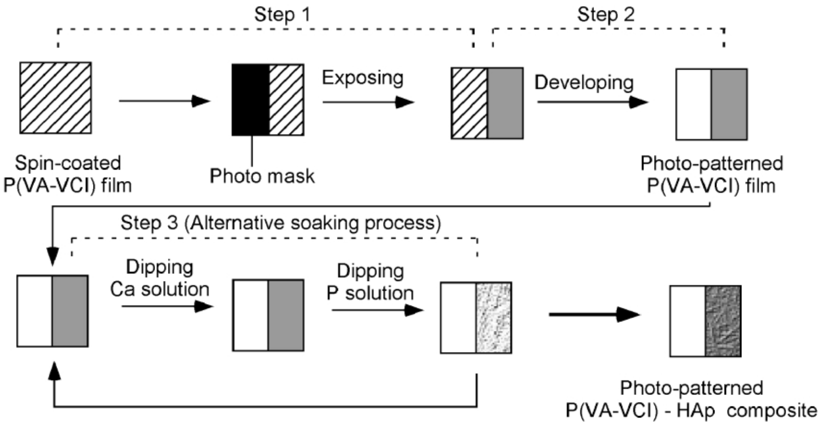

Fig. 1. Schematic presentation of preparation procedure of photo-patterned polymer–hydroxyapatite composite.

Step 1
A photo-patterned film with a substrate such as glass, metal, was soaked in 40 ml of $CaCl_2$ (200 mM) containing tris(hydroxylmethyl)aminomethane (50 mM) (Ca solution) at 37.5 °C for 2 h.
Step 2
The film was removed from the Ca solution, and was rinsed with excess distilled water to remove the attached excess Ca solution.
Step 3
The film was soaked in 40 ml of Na_2HPO_4 (120 mM) solution (P solution) at 37.5 °C for 2 h.
Step 4
The film was removed from the P solution and then rinsed with excess water.

By number of repeating times from Step 1 to 4, we controlled the amount of the deposited apatite in the film (Fig. 1).

2.5. Measurements

Infrared spectra of samples were recorded with an FTIR spectrophotometer (Shimadzu FTIR-8000A). ^{1}H-NMR measurements for samples were performed with an NMR spectrophotometer (JEOL EX-270). X-ray diffraction patterns of samples were collected with an X-ray diffractmeter (Shimadzu XD-1). Microphotographs of polymer films, polymer–apatite composites were taken with a CCD microscope system (Keyence VH-6300S).

3. Results and discussion

3.1. Photo-patterning of poly(vinylalcohol-co-vinyl trans-cinnamate) (P(VA-VCI))

Structure of P(VA-VCI) is shown in Fig. 2. The content of cinnamate moiety in the P(VA-VCI) was 79.4 mol%. We also prepared the P(VA-VCI) with various contents of cinnamate group. The P(VA-VCI) with 79.4 mol% cinnamate unit was suitable because of solubility of the polymer in the casting solution and homogeneity of the resulted film.

A P(VA-VCI) film on a glass plate was prepared by spin-coating technique. The typical film thickness was 6 μm. A patterned mask was closely contacted to the film surface. Then the P(VA-VCI) film on the glass plate with the mask was irradiated with a UV lamp ($\Lambda < 245$ nm) for 30 min and then dipped into a development solution (toluene, xylene, and 2-methoxyethyl acetate (1:1:1, by vol.) for 30 s. Fig. 3(a) shows the microscopic image of the photo-patterned film on the glass substrate. The boundary line between the substrate and the patterned P(VA-CVI) film was formed very clear. This indicates that a P(VA-CVI) film is photo-crosslinkable and can be used for photo-pattering process. A metal plate, such as titanium, stainless steel, aluminum, was also used as a spin-coating substrate. The spin-coated P(VA-CVI) films on the substrates were well adhered on them. The typical film thickness was 7–9 μm. The P(VA-CVI) films on the plates were also photo-crosslinkable. The boundary line between the substrate and the photo-patterned P(VA-CVI) film on the metal plate was also observed very clear. The results of contact angle measurements of the photo-patterned P(VA-VCI) films on various plates and the substrates are listed in Table 1.

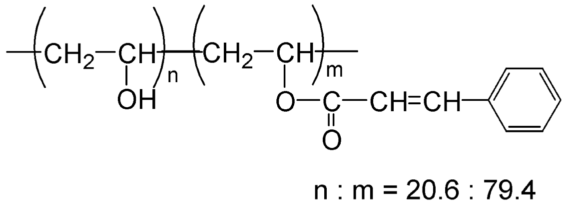

Fig. 2. Structure of poly(vinylalcohol-*co*-vinyl *trans*-cinnamate), P(VA-VCI).

The contact angle of the glass plate was 16.6°. The value of the P(VA-VCI) film on the glass was 65.3°. This suggests that the hydrophobicity of the P(VA-VCI) film is larger than that of the naked glass plate. Akashi et al. reported that the amount of HAp formed on/in PVA gel matrices increases with an increase in the swelling ratio of the gel [16]. They also reported that the deposition of HAp on the polyacrylamide (PAAm)-garfted polyethylene films is depended on the grafting densities of PAAm [9]. Thus, it is estimated that

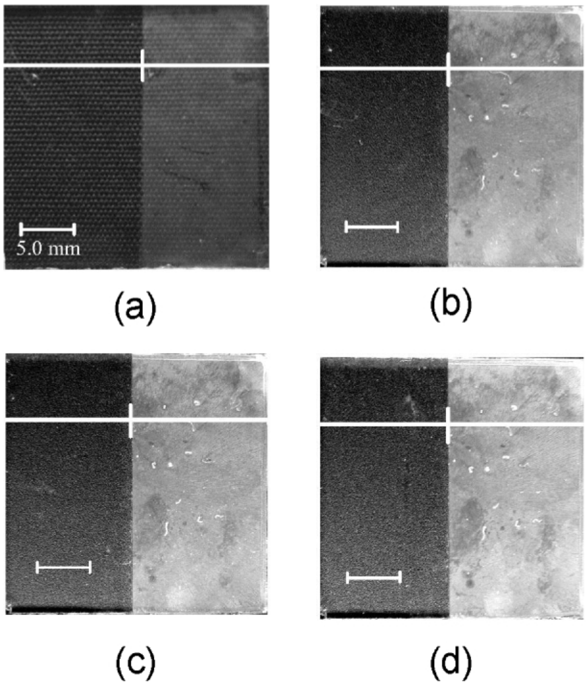

Fig. 3. Microscopic images of photo-patterned P(VA-VCI) film (a), alternative soaked P(VA-VCI) film (5 cycle) (b), alternative soaked P(VA-VCI) film (10 cycle) (c), and alternative soaked P(VA-VCI) film (15 cycle) (d). Left side from the center line is masked area and right side is exposed area. Scale bar in the photographs is 5 mm.

Table 1
Contact angle (H_2O) of photo-crosslinked P(VA-VCI) films on various substrates and of the naked substrates

Substrate	Contact angle (°)	
	Naked plate	Photo-crosslinked P(VA-VCI) film[a]
Glass	16.6	65.3
Aluminum	70.2	70.1
Stainless steel	63.9	67.6
Titanium	79.9	73.4

[a] Before photo-patterning process, contact angle of the P(VA-VCI) film was 69.2°.

the hydrophobicity of the matrix polymer (photo-crosslinked P(VA-VCI)) for the alternative soaking process gives large effect in the deposition behavior of HAp. However, the deposition of HAp in the P(VA-VCI) film was observed as mentioned below. It is shown that the deposition of HAp in the film is possible, if it is the hydrophobicity of this degree.

3.2. Alternative soaked photo-patterned poly(vinylalcohol-co-vinyl trans-cinnamate) (P(VA-VCI)) films

Alternative soaking process of a photo-patterned P(VA-VCI) film on a glass plate was performed. The microscopic images of the resulted film are shown in Fig. 3. Fig. 3(a) is the image of the photo-patterned film on the glass plate. The images of the alternative soaking processed film are shown in Fig. 3(b)–(d). The film became clouded homogeneously with increase in the number of soaking process cycle. The film thickness and weight also increased with the succession of the process. Fig. 4 shows the 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. After 15th soaking process, the thickness of the film increased from 6 to 11 μm. The thickness was measured at various points on the film. These values were almost equal each other (about ± 0.1 μm). This suggests that increase of the film thickness is uniformly and the film is homogeneous macroscopically.

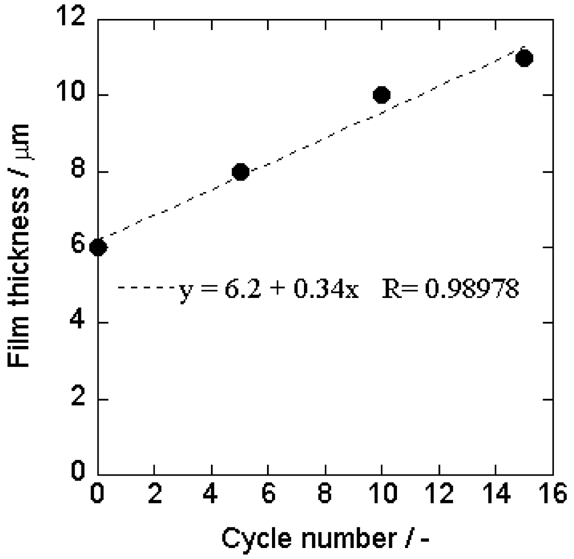

Fig. 4. Relationships between soaking cycle number and film thickness. Detail soaking condition was described in the experimental section.

Fig. 5 shows the X-ray diffraction patterns of the patterned P(VA-VCI) film (Fig. 5(a)) and the films processed with the alternative soaking treatment (Fig. 5(b)–(d)). Fig. 5(e) shows the X-ray diffraction pattern of hydroxyapatite in the JCPDS Card. Two large peaks at 25.8 and 32.8° are characteristic peaks for hydroxylapatite. The large two peaks were observed in the patterns in Fig. 5(b)–(d). This suggests that the white participates in the P(VA-CVI) film is hydroxylapatite. The peaks in the XRD charts were broad. This indicates that the deposited hydroxyapatite in the film is partially amorphous.

Fig. 6 shows IR 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 a hydroxyapatite (Fig. 6(b)). In Fig. 6(c) carbonate bands were observed at 879, 1415 and 1455 cm^{-1}. Thus, calcium carbonate is also 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).

Deposition of HAp in the P(VA-VCI) film on the metal plates was also observed. No dependence of the metal substrates on the deposition form of the HAp in the P(VA-VCI) film was detected on our experimental conditions. Copper was not suitable as a substrate in this study, because the alternative soaking process solution was corrosive for copper.

Deposition mechanism of HAp in a P(VA-VCI) film is as follows

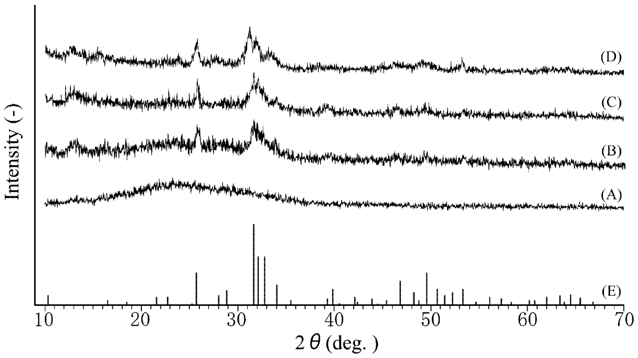

Fig. 5. X-ray diffraction patterns of P(VA-VCI) film (a), alternative soaked P(VA-VCI) film (5 cycle) (b), alternative soaked P(VA-VCI) film (10 cycle) (c), alternative soaked P(VA-VCI) film (15 cycle) (d), and peak positions from the JCPDS card of hydroxyapatite (e).

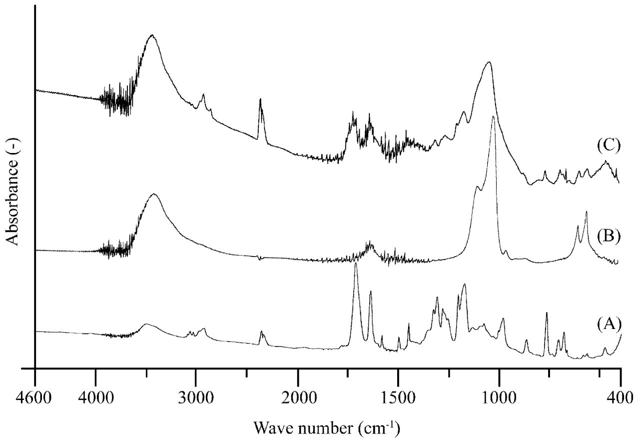

Fig. 6. Infrared spectra of photo-patterned P(VA-VCI) film (a), the surface (b) and the inside (c) of the alternative soaked P(VA-VCI) film (15 cycle).

Step 1
A photo-patterned P(VA-VCI) film with a substrate such as glass, metal, was soaked in the $CaCl_2$ aqueous solution at 37.5 °C for 2 h. Some amounts of calcium ions are trapped in the polymer film.
Step 2
The attached excess calcium ions on the film and the substrate uncovered with the polymer film are removed by the rinse process of the film with distilled water.
Step 3
The film was soaked in Na_2HPO_4 aqueous solution at 37.5 °C for 2 h. Some amounts of phosphate ions also trapped in the film containing calcium ions. Thus formation of HAp occurs in the polymer film.
Step 4
The attached excess phosphate ions on the film and the substrate uncovered with the polymer film are removed by the rinse process of the film with distilled water. Therefore, HAp is not formed in substrate which is uncovered with the polymer.

In conclusion, we demonstrated that it is possible to deposit hydroxyapatite in/on the photo-patterned P(VA-VCI) films using alternative soaking process. We are now studying the biological and other functions of the photo-patterned P(VA-VCI)–HAp composites. The details of those results will be reported in a subsequent paper.

References

[1] Loy DA. MRS Bull 2001;26(5):364–5.
[2] Yoshida W, Cohen YJ. J Membr Sci 2003;213(1–2):145–57.
[3] Nunes SP, Ruffmann B, Rikowski E, Vetter S, Richau K. J Membr Sci 2002;203:215–25.
[4] Leo CJ, Subba Rao GV, Chowdari BVR. Solid State Ionics 2002; 148(1–2):159–71.
[5] Kumar B, Rodrigues SJ, Spry RJ. Electrochim Acta 2002;47(8): 1275–81.
[6] Chung SH, Wang Y, Persi L, Croce F, Greenbaum SG, Scrosati B, Plichta E. J Power Sources 2001;97–98:644–8.
[7] Tanahashi M, Yao T, Kokubo T, Minoda M, Miyamoto T, Nakamura T, Yamamuro T. J Appl Biomater 1994;5(4):339–47.
[8] Tanahashi M, Yao T, Kokubo T, Minoda M, Miyamoto T, Nakamura T, Yamamuro T. J Biomed Mater Res 1995;29(3):349–57.
[9] Taguchi T, Muraoka Y, Matsuyama H, Kishida A, Akashi M. Biomaterials 2001;22:53–8.
[10] Ozawa N, Yao T. Solid State Ionics 2002;151:79–87.
[11] Tanahashi M, Yao TT, Kokubo M, Minoda T, Miyamoto T, Nakamura T, Yamamuro. J Am Ceram Soc 1994;77(11):2805–8.
[12] Tanahashi M, Yao T, Kokubo T, Minoda M, Miyamoto T, Nakamura T, Yamamuro T. J Mater Sci Mater Med 1995;6:319–26.
[13] Ozawa N, Yao T. J Biomed Mater Res 2002;62(4):579–86.
[14] Organic Synthesis, Col Vol 3, p. 714.
[15] Tsuda M. In: Millich F, Carraher CE Jr., editors. Interfacial synthesis, vol. II.; 1977. p. 471.
[16] Taguchi T, Kishida A, Akashi M. Chem Lett 1998;711–2.