Bonelike Apatite Formation on Ethylene-Vinyl Alcohol Copolymer Modified with a Silane Coupling Agent and Titania Solution

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シランカップリング剤及びチタニア溶液で処理したエチレン-ビニルアルコール共重合体上での骨類似アパタイト形成

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The surface of an ethylene-vinyl alcohol copolymer (EVOH) was modified with Ti-OH groups by a silane coupling treatment followed by treatment with a titania solution. The arrangement of the Ti-OH groups formed on EVOH was changed from amorphous into anatase structure by a subsequent HCl solution treatment. Bonelike nanoapatite was successfully formed on the surface-modified EVOH even in a form of fibers as well as plates within 2 d at 36.5°C in a simulated body fluid (SBF) with ion concentrations approximately equal to those of human blood plasma. The resultant nanoapatite-polymer fiber composite was similar to that of natural bone in its structure where apatite nanocrystals are deposited on fine organic fibers constituting a composite. Therefore, if the present surface modification can be applied to a three-dimensional fabric with a structure similar to the collagen fibers of natural bone, a composite with an analogous structure to that of natural bone would be obtained. The resultant composite is expected to exhibit mechanical properties analogous to those of natural bone, as well as bone-bonding ability, and hence be truly useful as a new bone substitute.

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1. Introduction

Natural bone is a composite in which inorganic apatite nanocrystals are deposited on organic collagen fibers woven into a three-dimensional structure.¹⁾ If synthetic organic polymer fibers are fabricated into a three-dimensional structure similar to that of the collagen fibers in natural bone, and bonelike apatite is deposited on their surfaces, a composite with an analogous structure to that of natural bone could be obtained. If the resultant composites exhibit analogous mechanical properties to those of natural bone,²⁾ and have bone-bonding ability through the bonelike apatite layer on its surface,^{3,4)} they can be truly useful as a bone substitute.

Although various attempts to prepare such a composite have been made, none of them have successfully produced a composite with bone-bonding ability and mechanical properties analogous to those of natural bone.^{5)–8)} For example, Shikunami and Kawarada prepared a three-dimensional polyethylene fabric, spray-coated with pure hydroxyapatite powder.⁷⁾ In this process, however, pure hydroxyapatite powder was physically deposited only on the outer surface of the fabric. Therefore, the stiffness of this fabric was not sufficient to allow the fabric to be used as a bone substitute under load-bearing conditions. Furthermore, pure hydroxyapatite is rather different from bone apatite in its composition and structure,⁹⁾ and hence requires a long period to bond to the bone.¹⁰⁾ In order to obtain bonelike apatite, apatite must be formed in a simulated body fluid (SBF)¹¹⁾

with ion concentrations approximately equal to those of human blood plasma.¹²⁾

The present authors have previously attempted to prepare the above-mentioned composite by a biomimetic process,^{13),14)} in which the surface of a polymeric material was modified with functional groups effective in inducing apatite nucleation, and then soaked in SBF. Si-OH groups were used as the functional groups effective in inducing apatite nucleation, since a pure silica gel rich in Si-OH groups forms bonelike apatite on its surface in SBF.^{15)–20)} An ethylene-vinyl alcohol copolymer (EVOH),²¹⁾ with high mechanical strength and good biocompatibility, was used as the polymeric material. The surface modification of EVOH with Si-OH groups was carried out by treatment with a silane coupling agent, namely, 3-isocyanatopropyltriethoxysilane (OCN(CH₂)₃Si(OC₂H₅)₃; IPTS), and a calcium silicate solution. The surface-modified specimen formed apatite on its surface in SBF within 2 d at 36.5°C. In this process, however, a calcium-containing silica gel layer formed on the specimen is liable to dissolve into the surrounding fluid over a long period.

Recently, it has been shown that not only Si-OH, but also other functional groups such as Ti-OH,^{22)–24)} Ta-OH,²⁵⁾ Nb-OH²⁶⁾ and Zr-OH²⁷⁾ are effective for apatite nucleation in SBF. Among them, Ti-OH groups in a titania gel with an anatase structure have been reported to be remarkably effective in inducing apatite nucleation in SBF.²⁴⁾ In addi-

tion, a titania gel would be stable in the body environment for a long period, since the solubility of a titania gel is much lower than that of a silica gel.²⁸⁾

In the present study, the surfaces of an EVOH plate and a knitted yarn of EVOH fibers were modified with Ti-OH groups by treatment with a silane coupling agent and a titania solution. The arrangement of the Ti-OH groups formed on EVOH was changed into anatase structure by subsequent HCl solution treatment. The apatite-forming ability of the surface-modified specimen was examined in SBF.

2. Experimental

2.1 Preparation of specimens

An EVOH plate, 1 mm in thickness, was prepared by hot-pressing pellets of EVOH with quoted ethylene content of 32 mol%, generously supplied by Kuraray Co., Ltd., Kurashiki, Japan, at 200°C. The EVOH plate was cut into square pieces, 10 × 10 mm² in size, and then abraded with a #400 diamond pad. Knitted yarn of EVOH made of 420 fibers with a diameter of 3 μm and with quoted ethylene content of 32 mol%, was generously supplied by Kuraray Co., Ltd., Kurashiki, Japan. A specimen of the knitted yarn of EVOH fibers was cut into square pieces, 10 × 10 mm² in size. Specimens of both the EVOH plate and the knitted yarn of EVOH fibers were ultrasonically washed with acetone and 2-propanol, and then dried at 100°C under vacuum for 24 h.

2.2 Silane coupling treatment

The specimen was soaked in a solution with a mass ratio of IPTS (Chisso Co., Tokyo): toluene (Wako Pure Chemical Industries Ltd., Osaka): dibutyltin dilaurate (Nacalai Tesque Inc., Kyoto) of 1.0 : 1.0 : 0.005 at 50°C for 6 h in a nitrogen atmosphere. The specimen was removed from the solution, washed sequentially with tetrahydrofuran, 2-propanol and toluene, and then dried at room temperature for 24 h under vacuum (denoted as EI). The specimen was subsequently soaked in 0.05 M HCl aqueous solution at 40°C for 12 h, washed with ultra-pure water, and then dried at room temperature for 24 h under vacuum (denoted as EIH).

2.3 Titania solution treatment

A titania solution with a molar ratio of Ti(OiC₃H₇)₄ : H₂O : HNO₃ : C₂H₅OH of 1.0 : 1.0 : 0.1 : 9.25 was prepared by stirring a mixture of Ti(OiC₃H₇)₄, ultra-pure water, ethyl alcohol, and 60 mass% HNO₃ aqueous solution (Nacalai Tesque Inc., Kyoto) at 0°C. The specimen of EIH was soaked in the titania solution at 25°C for 24 h, withdrawn from the solution at a rate of 2 cm/min, and then heated at 100°C for 10 min. The specimen was then dipped again into the solution, immediately withdrawn at the same rate, and then heated at 100°C for 10 min. After this dipping, withdrawing and heating process was repeated four times, the specimen was finally heated at 100°C for 24 h (denoted as EIHT).

2.4 HCl solution treatment

The specimen of EIHT was subsequently treated with ultra-pure water or HCl aqueous solutions at various concentrations up to 1.00 M at 80°C for various periods up to 8 d. The specimen, removed from the solution, was washed with ultra-pure water at 40°C for 12 h.

2.5 Soaking in SBF

The surface-modified specimen was soaked in 30 ml of SBF¹¹⁾ at pH 7.40 and with ion concentrations (Na⁺ 142.0, K⁺ 5.0, Mg²⁺ 1.5, Ca²⁺ 2.5, Cl⁻ 147.8, HCO₃⁻ 4.2, HPO₄²⁻ 1.0, SO₄²⁻ 0.5 mM) approximately equal to those of human blood plasma at 36.5°C for various periods up to 14 d. The SBF was prepared by dissolving reagent-grade chemicals of NaCl, NaHCO₃, KCl, K₂HPO₄ · 3H₂O, MgCl₂ · 6H₂O, CaCl₂, and Na₂SO₄ (Nacalai Tesque Inc., Kyoto) in ultra-pure

water, and buffering at pH 7.40 with tris(hydroxymethyl) aminomethane ((CH₂OH)₃CNH₂) and 1.00 M HCl aqueous solution (Nacalai Tesque Inc., Kyoto) at 36.5°C.²⁹⁾ After a given soaking period, the specimen was removed from the fluid, gently washed with ultra-pure water, and then dried at room temperature for 24 h.

2.6 Surface characterization

The surface structures of the specimens were examined by using an X-ray photoelectron spectrometer (XPS; MT-5500, ULVAC-PHI Co., Ltd., Chigasaki) with Mg Kα radiation, a thin-film X-ray diffractometer (TF-XRD; RINT2500, Rigaku Co., Tokyo) with Cu Kα radiation, and a field emission scanning electron microscope (FE-SEM; S-4700, Hitachi Ltd., Tokyo). The photoelectron take-off angle was set at 45° for XPS, and the glancing angle was set at 1° against the direction of the incident beam with a thin film attachment for TF-XRD.

3. Results

Survey and C_{1s} XPS spectra are shown in Fig. 1(a) and (b), respectively, for the surfaces of the EVOH, EI and EIH plates. After the silane coupling treatment (EI), peaks ascribed to N_{1s}, Si_{2s} and Si_{2p} appeared on the survey spectra (see Fig. 1(a)). On the C_{1s} spectra in Fig. 1(b), a peak ascribed to C=O appeared after the silane coupling treatment (EI). These results indicate that the surface of the EVOH plate reacted with an isocyanato group of IPTS during the silane coupling treatment, causing Si-OC₂H₅ groups to be introduced onto the EVOH surface as shown in the following equation,³⁰⁾

where *R* represents a carbon atom on the EVOH surface. When the EI plate was subsequently treated with an HCl solution (EIH), a shoulder ascribed to C-O disappeared in the C_{1s} spectra (see Fig. 1(b)). This result indicates that Si-OC₂H₅ groups on the specimen were hydrolyzed into Si-OH groups due to the catalytic effect of HCl.

Figure 2 shows XPS survey spectra (a), and TF-XRD patterns (b), of surfaces of the EIHT plates, untreated and treated with ultra-pure water or HCl solutions with various concentrations up to 1.00 M for 5 d. On the XPS spectra in Fig. 2(a), peaks ascribed to Ti_{2p} and Ti_{3p} were observed for the untreated EIHT plate, i.e., the specimen as treated with a titania solution. It can be seen from this result that a titania layer was successfully formed on the specimen after the titania solution treatment. The peak intensity of C_{1s} in the spectra decreased with increasing HCl concentration up to 0.10 M in subsequent treatment with ultra-pure water or HCl solutions. This indicates that the number of alkoxy groups in the titania layer decreased with increasing HCl concentration up to 0.10 M in the treatment. The decrease in the number of alkoxy groups is attributed to a hydrolysis reaction of alkoxy groups in the titania layer to form Ti-OH groups, catalyzed by HCl. For the specimen treated with 1.00 M HCl solution, peaks ascribed to Ti_{2p} and Ti_{3p} were not observed, whereas those ascribed to N_{1s}, Si_{2s} and Si_{2p} were observed, giving a spectrum quite similar to that of the EIH plate, i.e., the specimen before the titania solution treatment (see Fig. 1(a)). This result suggests that the titania layer on the specimen dissolved into 1.00 M HCl solution by the HCl solution treatment.

On the TF-XRD patterns in Fig. 2(b), a peak ascribed to anatase was detected for the EIHT plate treated with ultra-pure water, 0.01 M or 0.10 M HCl solution for 5 d, but not detected for the untreated EIHT plate. This can be interpreted as the structure of the titania layer on the specimen being transformed from an amorphous form into anatase by

Fig. 1. Survey (a) and C_{1s} (b) XPS spectra of surfaces of the EVOH, EI and EIH plates.

Fig. 2. XPS survey spectra (a) and TF-XRD patterns (b) of surfaces of the EIHT plates, untreated and treated with ultra-pure water or HCl solutions with various concentrations up to 1.00 M for 5 d.

treatment with ultra-pure water or HCl solutions. The peak intensity of anatase increased with increasing HCl concentration up to 0.10 M in the treatment. This indicates that the transformation of amorphous titania into anatase was promoted by HCl through its catalytic effect on a hydrolysis reaction of alkoxy groups to form Ti-OH groups in the titania layer. However, no peak ascribed to anatase was observed for the specimen treated with 1.00 M HCl solution.

This might be because the titania layer on the specimen dissolved into 1.00 M HCl solution by the HCl solution treatment, as noted above from the XPS result.

Figure 3 shows TF-XRD patterns of surfaces of the EIHT plates, untreated and treated with ultra-pure water or HCl solutions with various concentrations up to 1.00 M for 5 d, and subsequently soaked in SBF for 14 d. The untreated EIHT plate and the EIHT plate treated with ultra-pure

water or 1.00 M HCl solution, did not form apatite on their surfaces in SBF even after 14 d. In contrast, the EIHT plate treated with 0.01 M or 0.10 M HCl solution formed apatite on its surface within 14 d. The peak intensity of apatite was higher for the specimen treated with 0.10 M HCl solution than that treated with 0.01 M HCl solution.

Fig. 3. TF-XRD patterns of surfaces of the EIHT plates, untreated and treated with ultra-pure water or HCl solutions with various concentrations up to 1.00 M for 5 d, and subsequently soaked in SBF for 14 d.

XPS survey spectra (a), and TF-XRD patterns (b), of surfaces of the EIHT plates, untreated and treated with 0.10 M HCl solution for various periods up to 8 d, are shown in Fig. 4. On the XPS spectra in Fig. 4(a), peaks ascribed to Ti_{2p} and Ti_{3p} were observed for all of the specimens. The peak intensity of C_{1s} on the spectra decreased with increasing period of HCl solution treatment up to 8 d, indicating that the number of alkoxy groups in the titania layer decreased with increasing period of treatment. This is because the alkoxy groups in the titania layer were hydrolyzed into Ti-OH groups by the HCl solution treatment.

On the TF-XRD patterns in Fig. 4(b), the peak intensity of anatase increased with increasing period of HCl solution treatment up to 8 d. This indicates that the amount of deposited anatase on the specimen increased with increasing period of HCl solution treatment up to 8 d.

Figure 5 shows TF-XRD patterns of surfaces of the EIHT plates, untreated and treated with 0.10 M HCl solution for various periods up to 8 d, and subsequently soaked in SBF for 2 d (a), 7 d (b), or 14 d (c). The EIHT plates treated with 0.10 M HCl solution for 3, 5 and 8 d formed apatite on their surfaces within 7, 2 and 2 d, respectively. Among the specimens soaked in SBF for 2 d, the specimen treated for 8 d gave a higher peak intensity of apatite than the specimen treated for 5 d (see Fig. 5(a)).

FE-SEM photographs of surfaces of the plates of EVOH (a), EIH (b), EIHT treated with 0.10 M HCl solution for 8 d (c), and EIHT treated with 0.10 M HCl solution for 8 d and then soaked in SBF for 2 d (d), are shown in Fig. 6. The surface morphologies of the EVOH and EIH plates were almost the same (see Fig. 6(a) and (b)). This is attributed to the extremely thin IPTS layer formed on the EIH plate.^{13),14)} After subsequent treatment with titania and HCl solutions, a new layer was formed on the surface of the specimen (see Fig. 6 (c)). This layer should be a titania layer with an anatase structure, according to the XPS and TF-XRD results shown in Fig. 4. When this specimen was subsequently soaked in SBF for 2 d, a layer consisting of

Fig. 4. XPS survey spectra (a) and TF-XRD patterns (b) of surfaces of the EIHT plates, untreated and treated with 0.10 M HCl solution for various periods up to 8 d.

Fig. 5. TF-XRD patterns of surfaces of the EIHT plates, untreated and treated with 0.10 M HCl solution for various periods up to 8 d, and subsequently soaked in SBF for 2 d (a), 7 d (b), or 14 d (c).

Fig. 6. FE-SEM photographs of surfaces of the plates of EVOH (a), EIH (b), EIHT treated with 0.10 M HCl solution for 8 d (c), and EIHT treated with 0.10 M HCl solution for 8 d and then soaked in SBF for 2 d (d).

particles with a diameter of approximately $3\ \mu\text{m}$ was observed on the specimen (see Fig. 6(d)). This layer should be apatite, as indicated from the TF-XRD result in Fig. 5. An FE-SEM photograph at higher magnification showed the nano-sized particles constituting this apatite (see Fig. 6

(d) right).

Figure 7 shows FE-SEM photographs of surfaces of the knitted yarn of fibers of EVOH (a), EIH (b), EIHT treated with 0.10 M HCl solution for 8 d (c), and EIHT treated with 0.10 M HCl solution for 8 d and then soaked in SBF for 2 d

Fig. 7. FE-SEM photographs of surfaces of the knitted yarn of fibers of EVOH (a), EIH (b), and EIHT treated with 0.10 M HCl solution for 8 d (c), and EIHT treated with 0.10 M HCl solution for 8 d and then soaked in SBF for 2 d (d).

(d). The specimen changed its surface morphology in the same manner as the EVOH plate shown in Fig. 6. A dense apatite layer consisting of nano-sized particles was observed on the surface of the specimen soaked in SBF for 2 d (see Fig. 7(d)).

4. Discussion

It was found from the results obtained in the present study that the apatite-forming ability of surface-modified EVOH was significantly dependent on the HCl concentration and period of treatment with HCl solutions for the EIHT plate. As shown in Fig. 3, both the untreated EIHT plate and the EIHT plate treated with ultra-pure water did not form apatite on their surfaces in SBF, even after 14 d. This might be attributed to a small number of Ti-OH groups on the specimen because of remaining unhydrolyzed alkoxy groups, and the amorphous structure of the titania layer on the specimen, which is not effective in inducing apatite nucleation.²⁴⁾ The former is supported by the XPS result (see Fig. 2(a)), and the latter is supported by the TF-XRD result (see Fig. 2(b)).

The EIHT plate treated with 1.00 M HCl solution also formed no apatite on its surface in SBF even after 14 d, as shown in Fig. 3. This can be explained as follows. The titania layer that formed on the EIHT plate was originally an amorphous layer containing a large number of alkoxy groups. It is thought that such a layer is unstable, and hence easily dissolved into 1.00 M HCl solution during the HCl solution treatment. This was confirmed by the observation that the surface composition of the specimen treated with 1.00 M HCl solution was quite similar to that of the EIH plate, i.e., the specimen as treated with IPTS before the titania solution treatment (see Fig. 1(a) and Fig. 2(a)). The Si-OH groups remaining on the specimen after the HCl solution treatment are not effective for apatite nucleation in

SBF.^{8),13)}

In contrast, the EIHT plate treated with 0.01 M HCl solution for 5 d, and the EIHT plate treated with 0.10 M HCl solution for 3, 5 or 8 d, formed apatite on their surfaces in SBF within 14 d, as shown in Figs. 3, 5 and 6. The apatite-forming ability of the specimen was improved with increasing HCl concentration up to 0.10 M in the HCl solution treatment (see Fig. 3) and increasing period of the treatment up to 8 d (see Fig. 5). This is explained as follows. During the HCl solution treatment, the titania layer, formed on the specimen by the titania solution treatment, transforms its structure from amorphous to anatase, through hydrolysis of alkoxy groups existing in the titania layer into Ti-OH groups, and subsequent condensation of the Ti-OH groups to form a Ti-O-Ti network. This hypothesis is supported by the XPS and TF-XRD results shown in Figs. 2 and 4. The HCl catalyzes hydrolysis of alkoxy groups to form many Ti-OH groups and promotes the transformation of amorphous titania into anatase. Ti-OH groups have been believed to be effective in inducing apatite nucleation in SBF, from the experimental fact that a titania gel with an anatase structure rich in Ti-OH groups forms apatite on its surface in SBF, whereas an anatase single crystal with little Ti-OH groups does not.³¹⁾ However, all kinds of Ti-OH groups are not equally effective in inducing apatite nucleation. The Ti-OH groups in an anatase structure are more effective in inducing apatite nucleation than those in amorphous structure.²⁴⁾ Therefore, both the increase in number of Ti-OH groups and the transformation of the titania from amorphous into anatase on the specimen, caused by the increase in HCl concentration and period in the HCl solution treatment, are responsible for the improvement of the apatite-forming ability of the specimen. Once the apatite nuclei are formed, they grow spontaneously by consuming calcium and phosphate ions from SBF, since the SBF is

highly supersaturated with respect to apatite.³²⁾

The EIHT plate, which was treated with 0.10 M HCl solution for 8 d, showed the best apatite-forming ability among all of the examined specimens. In fact, a dense layer consisting of apatite nanocrystals was successfully formed on the EIHT plate, which was treated with 0.10 M HCl solution for 8 d and then soaked in SBF for 2 d (Fig. 6(d)). The apatite layer formed on this plate adhered to the specimen surface so strongly that the apatite layer was not peeled off from the surface of the specimen, even by a tape-detaching test using Scotch® tape. The same kind of nanoapatite layer was successfully formed even on the surface of individual EVOH fiber constituting the knitted yarn, which was surface-modified and soaked in SBF for 2 d (Fig. 7(d)). This composite structure is similar to that of natural bone, in which apatite nanocrystals are deposited on fine organic collagen fibers.¹⁾ Therefore, if the present surface modification can be applied to a three-dimensional fabric with a structure similar to that of the collagen fibers in natural bone, a nanoapatite-organic polymer fiber composite with an analogous structure to that of natural bone could be obtained. The resultant composite could have great potential as a bone substitute, since it is expected to exhibit mechanical properties analogous to those of natural bone,²⁾ as well as high bone-bonding ability through a bonelike apatite layer on its surface.^{3),4)}

5. Conclusion

Bonelike apatite was successfully formed on a knitted yarn of EVOH fibers as well as on an EVOH plate in SBF at 36.5°C within only 2 d, when the surface of the EVOH was previously modified with Ti-OH groups with an anatase structure. The present surface modification of EVOH would be useful for obtaining a nanoapatite-organic polymer fiber composite with an analogous structure to that of natural bone. The resultant composite could be a new bone substitute with mechanical properties and bone-bonding ability analogous to those of natural bone.

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