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Formation of biologically active bone-like apatite on metals and polymers by a biomimetic process[1]

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Abstract

Some ceramics bond to living bone through a bone-like apatite layer which is formed on their surfaces in the living body. The formation of the apatite layer is induced by Si–OH or Ti–OH groups on their surfaces. These findings provide us with a biomimetic process with which to form a bone-like apatite layer on metals and organic polymers. Titanium metal and its alloys form a thin alkali titanate layer on their surfaces when they are subjected to alkaline solution and heat treatments. Thus, treated metals form a dense, uniform bone-like apatite layer on their surfaces in acellular simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma. Organic polymers form apatite nuclei on their surfaces when they are placed on $CaO–SiO_2$-based glass grains soaked in SBF. Thus, treated polymers form a dense, uniform bone-like apatite layer on their surfaces in SBF or other solutions highly supersaturated with respect to the apatite. The thickness of the apatite layer increases with increasing soaking time in the solution. The composition and structure of the apatite can be controlled by ion concentrations in the solution. The adhesive strengths of the apatite layer to the metal substrates are very high, and those to the polymer substrates can be considerably increased by glow discharge pretreatment of the substrates.

Keywords: Apatite; Biomimetic process; Metals; Polymers

1. Introduction

Generally, artificial materials implanted into bone defects are encapsulated by fibrous tissue isolated from the surrounding bones. It has been shown, however, during the last 25 years that several kinds of ceramics bond to living bone without forming fibrous tissue around them. Some of them e.g., Bioglass in the system $Na_2O–CaO–$

[1] Dedicated to Professor Hiroshi Suga.

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SiO_2–P_2O_5, sintered hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and the glass-ceramic A-W containing apatite and wollastonite ($CaO{\cdot}SiO_2$) are already used clinically as important bone-repairing materials [1]. Even the glass-ceramic A–W, with the highest mechanical strength, however, cannot be used under high load-bearing conditions, such as in femoral and tibial bones, since its fracture toughness is lower and elastic modulus higher than those of human cortical bone.

However, our fundamental understanding of the bone-bonding mechanism of these ceramics is considerably advanced. It is known for various kinds of glasses and glass-ceramics that the essential requirement for them to bond to living bone is the formation of a biologically active bone-like apatite layer on their surfaces in the body [2, 3]. This bone-like apatite layer can be reproduced on their surfaces even in acellular simulated body fluid [4]. The mechanism of the formation of the bone-like apatite on their surfaces is, therefore, understood in terms of surface chemistry. These findings enable us to form the bone-like apatite layer even on surfaces of metals and organic polymers in vitro. Such metals and polymers grown with apatite on their surfaces are believed to be useful as bone-repairing materials even under high load-bearing conditions, because they have high fracture toughness and/or low elastic modulus, as well as high bioactivity.

In the present paper, the formation of a biologically active bone-like apatite layer on metals and organic polymers by a biomimetic process, which is based on the mechanism of formation of a bone-like apatite layer on the surfaces of CaO-SiO_2-based glasses and glass-ceramics in the living body, is reviewed.

2. Mechanism of bone-like apatite formation in the living body

All known kinds of bioactive glasses and glass-ceramics bond to living bone through an apatite layer which is formed on their surfaces in the living body, as shown in Fig. 1 [5]. The apatite layer can be reproduced on their surfaces in acellular simulated body fluid (SBF) with ion concentrations (Na^+, 142.0; K^+, 5.0; Mg^{2+}, 1.5; Ca^{2+}, 2.5; Cl^-, 148.0; HCO_3^-, 4.2; HPO_4^{2-}, 1.0; SO_4^{2-}, 0.5 mM) nearly equal to those of human blood plasma [6] and identified as a layer of a carbonate-containing hydroxyapatite with small crystallites and defective structure similar to apatite in bone [7].

The mechanism of the bone-like apatite formation on the surfaces of CaO–SiO_2-based glasses and glass-ceramics in the living body is explained as follows [8]. The calcium ion released from them increases the ionic activity product of the apatite in the surrounding fluid, and hydrated silica on their surfaces provides favorable sites for apatite nucleation, as shown in Fig. 2. Consequently, a lot of apatite nuclei are formed on their surfaces. Once the apatite nuclei are formed, they grow spontaneously by consuming the calcium and phosphate ions from the surrounding fluid, since body fluid is already highly supersaturated with respect to apatite even under normal conditions.

The catalytic effect of hydrated silica for apatite nucleation is demonstrated by the observation that even pure silica gel prepared by a sol-gel method forms bone-like apatite on its surface in SBF, as shown in Fig. 3 (left) [9]. In addition to silica gel, titania gel prepared by a sol-gel method was also found to induce apatite nucleation on it in

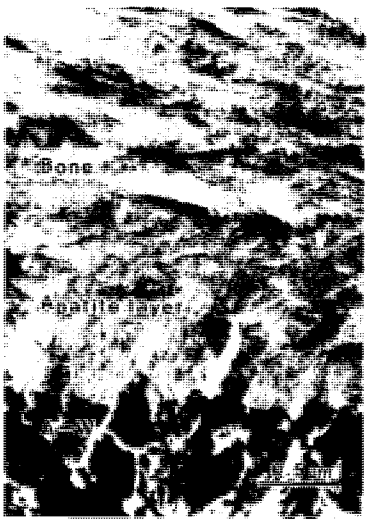

Fig. 1. Transmission electron micrograph of an interface between glass-ceramic A–W and living bone.

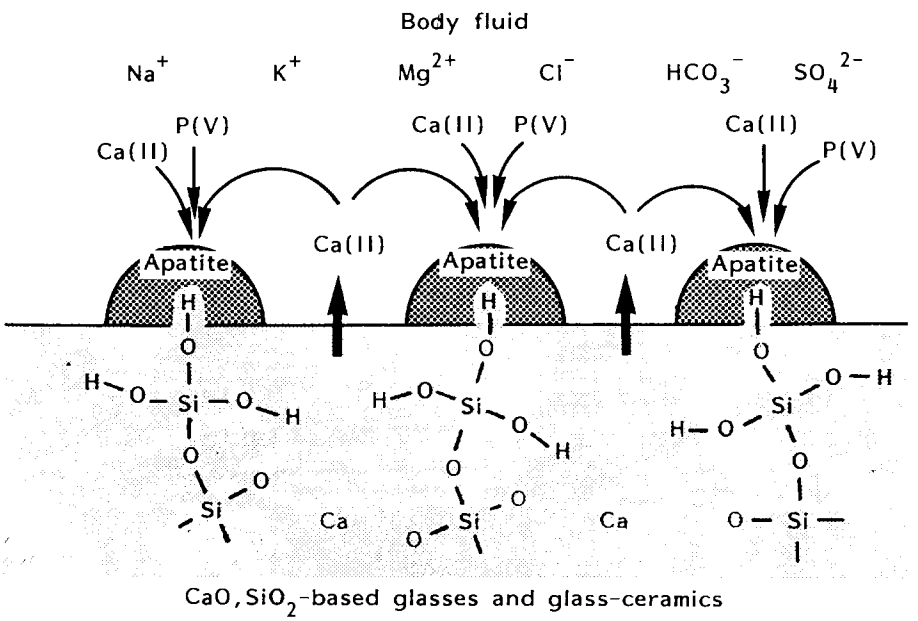

Fig. 2. Mechanism of apatite formation on the surfaces of $CaO\text{-}SiO_2$-based glasses and glass-ceramics.

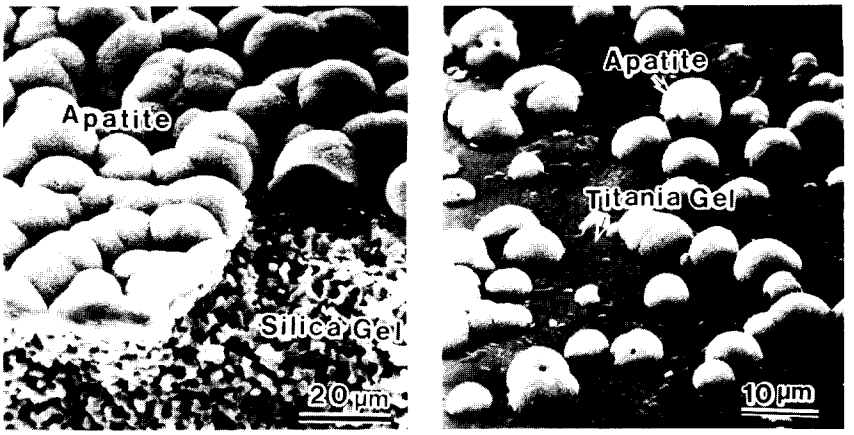

Fig. 3. Apatite formation on silica gel (left) and titania gel (right) in SBF.

SBF, as shown in Fig. 3 (right) [10]. These findings provide a biomimetic method for forming a bone-like apatite layer on metals and organic polymers.

3. Formation of bone-like apatite on metals

Generally, surfaces of titanium metal and its alloys are covered with a thin passive titanium oxide layer. In alkali solution, however, even this passive layer dissolves by the following reaction [11]

$$TiO_2 + OH^- \rightarrow HTiO_3^- \quad (1)$$

Simultaneously, Ti metal is hydrated by the following reactions

$$Ti + 3OH^- \rightarrow Ti(OH)_3^+ + 4e^- \quad (2)$$

$$Ti(OH)_3^+ + e^- \rightarrow TiO_2 \cdot H_2O + 1/2H_2\uparrow \quad (3)$$

$$Ti(OH)_3^+ + OH^- \rightarrow Ti(OH)_4 \quad (4)$$

$$TiO_2 \cdot nH_2O + OH^- \rightarrow HTiO_3^- \cdot nH_2O \quad (5)$$

These negatively charged hydrated titania species react with positively charged alkali ions to produce an alkali titanate hydrogel layer on the surfaces of Ti metal and its alloys, as shown in Fig. 4. Actually, when Ti metal and its alloys, such as Ti-6Al-4V, Ti–15Mo–5Zr–3Al and Ti–6Al–2Nb–Ta alloys, are exposed to 10 M NaOH or KOH aqueous solution at 60°C for 24 h, a gel layer characterized by a halo pattern in the 2θ range 25–30° on thin-film X-ray diffraction patterns of their surfaces, is formed as shown in Fig. 5 [12]. This gel layer is unstable mechanically as well as chemically. It is, however, stabilized as an amorphous and crystalline alkali titanate layer by a heat treatment at around 600 °C.

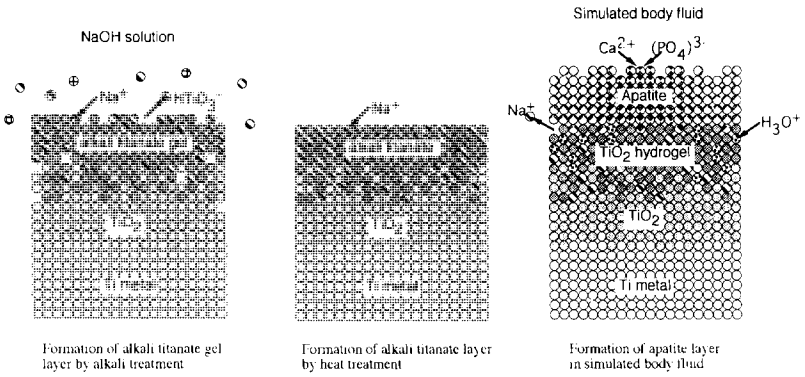

Fig. 4. Schematic representation of the chemical reaction on the surface of Ti metal on alkali and subsequent heat-treatment and soaking in SBF.

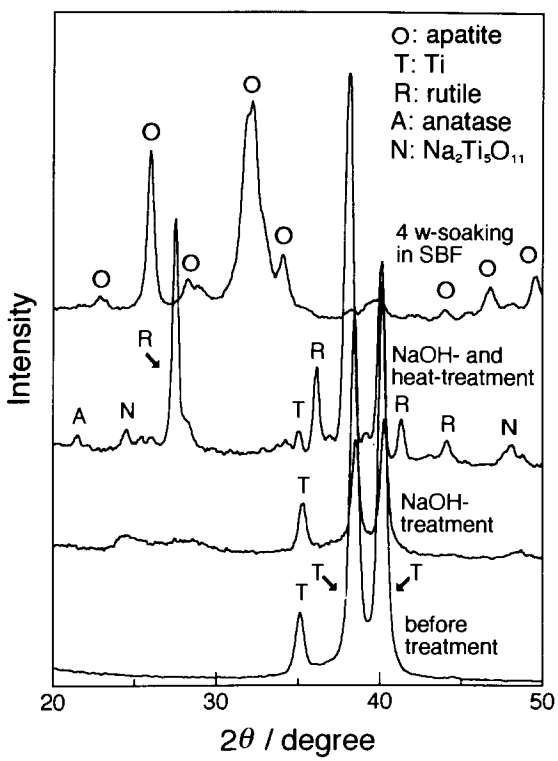

Fig. 5. Thin-film X-ray diffraction patterns on the surface of Ti metal, treated with 10 M NaOH solution at 60°C for 24 h, then heat-treated at 600°C for 1 h and subsequently soaked in simulated body fluid for 4 weeks.

When metals treated in this way are soaked in SBF, the alkali ion in the alkali titanate layer exchanges with the hydronium ion in the fluid, to increase the ionic activity product of the apatite in the fluid by increasing pH, as shown in Fig. 6. Simultaneously, hydrated titania is formed on their surfaces, and induces the apatite

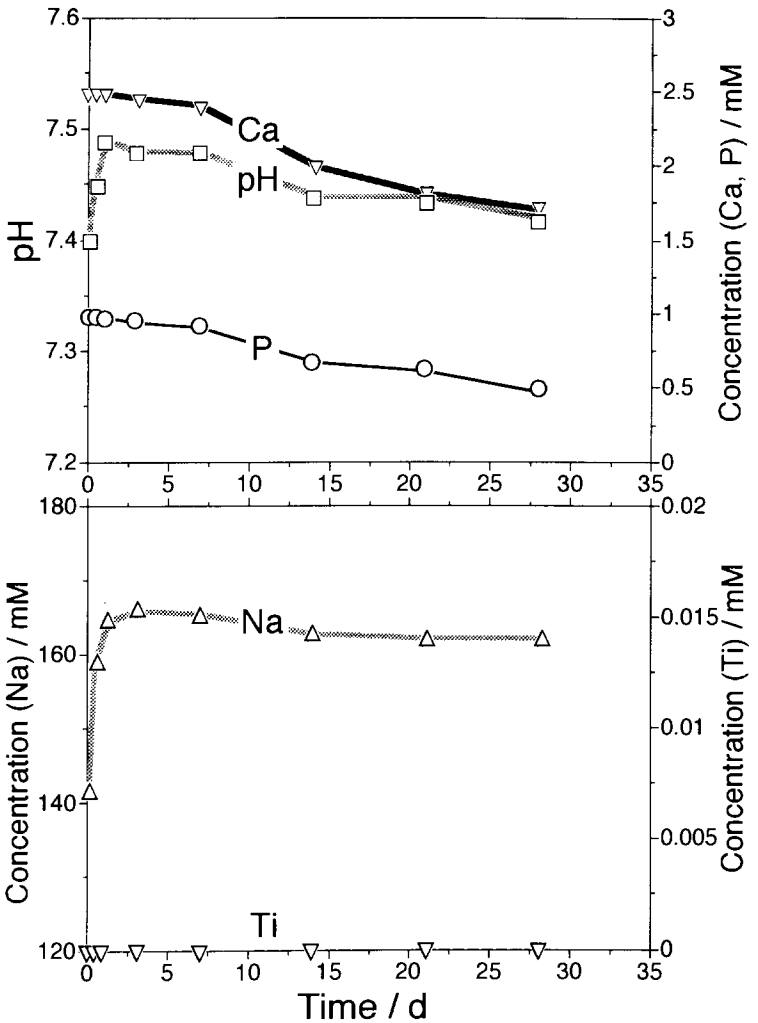

Fig. 6. Variation in element concentrations of SBF with soaking of alkali- and heat-treated Ti metal.

nucleation. Consequently, a lot of apatite nuclei are formed on their surfaces. These nuclei grow spontaneously by consuming the calcium and phosphate ions from the surrounding fluid, as shown by the decrease in Ca and P concentrations in Fig. 6. As a result, a dense, uniform bone-like apatite layer is formed on the surfaces of Ti metal and its alloys, as shown in Figs. 4, 5 and 7. The apatite layer thus formed is tightly bonded to the substrates, since it is integrated with the substrates through the hydrated titania and titanium oxide layer, concentrations of which are gradually changed from the outer surfaces to the inner substrates.

It is expected that the same reaction forming the bone-like apatite could occur on surfaces of alkali and heat-treated metals even in the living body. This means that

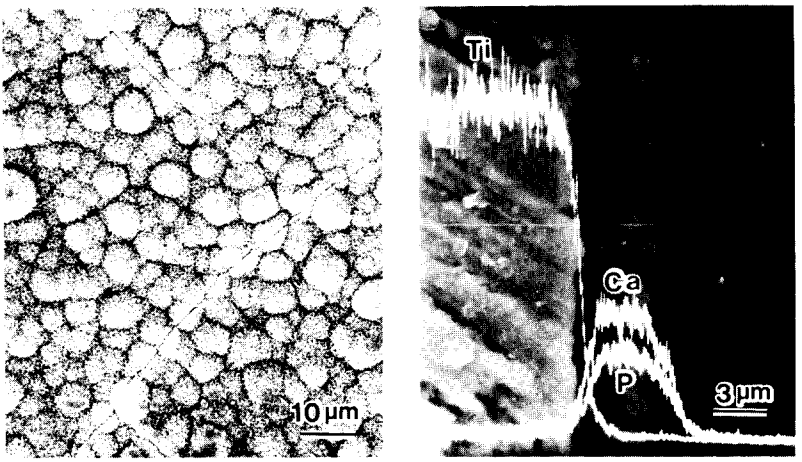

Fig. 7. SEM-EDX photograph of the surface (left) and cross-section (right) of alkali- and heat-treated Ti metal soaked in SBF for 4 weeks.

bioactive metals are obtained by alkali and heat treatments of Ti metal and its alloys. Bioactive metals thus obtained are believed to be useful as artificial bones, even under high load-bearing conditions, since they exhibit high fracture toughness as well as high bioactivity.

4. Formation of bone-like apatite on polymers

Organic polymers can also form a bone-like apatite layer on their surfaces in SBF or solutions supersaturated with respect to the apatite, if their surfaces are previously subjected to an appropriate treatment. For example, if polymer substrates are placed on granular particles, 150–300 μm in size, of a CaO–SiO_2-based glass (e.g. MgO, 4.6; CaO, 44.7; SiO_2, 34.0; P_2O_5, 16.2; and CaF_2 0.5 wt%) soaked in SBF, as shown in Fig. 8, silicate ion released from the glass particles is adsorbed on their surfaces and induces apatite nucleation there. Then, if they are soaked in another solution highly supersaturated with respect to the apatite, e.g. a solution (1.5 SBF) with ion concentrations 1.5 times those of SBF, the apatite nuclei grow on their surfaces in situ by consuming the calcium and phosphate ions from the surrounding fluid [13]. As the period of the first treatment increases, the number of apatite nuclei increases and hence a continuous apatite layer is formed during the second treatment, as shown in Fig. 9.

The induction period for apatite nucleation, which is defined as the period of the first treatment required for forming a continuous apatite layer, is 1 day for most of the common polymers such as poly(ethylene terephthalate) (PET), poly(methyl methacrylate) (PMMA), polyether sulfone (PESF), polyamide 6 (Nylon 6), polyethylene (PE) and poly(tetrafluoro ethylene) (PTFE) [13]. If these substrates are previously subjected to glow discharge treatment in O_2 gas for 30 s, the induction period is decreased down

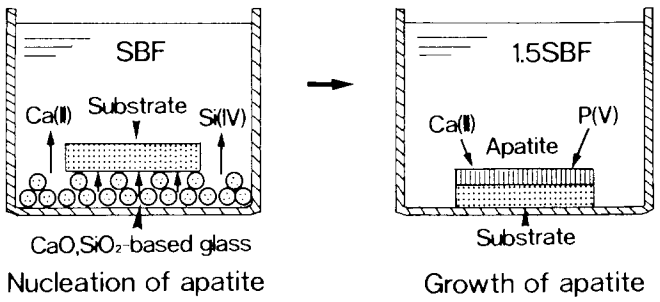

Fig. 8. Biomimetic process for apatite formation on polymer substrates.

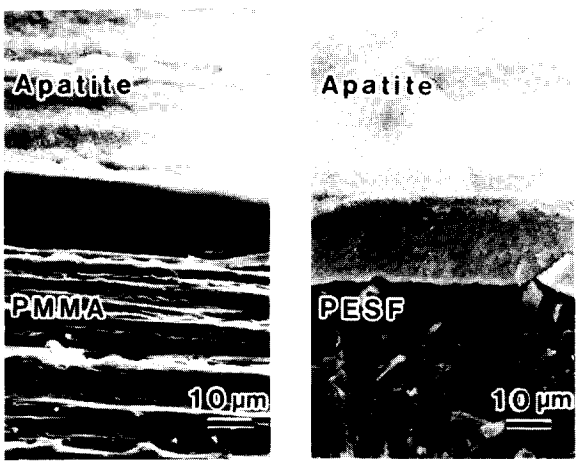

Fig. 9. Dense and uniform apatite layer formed on some polymer substrates.

to 6 h, since polar groups, such as carbonyl, ester, hydroxyl and carboxyl groups, are formed on their surfaces by the glow discharge treatment, as shown in Fig. 10, and effectively trap the silicate ion [14].

The thickness of the apatite layer increases linearly with increasing period of the second treatment as far as the calcium and phosphate ions are supplied, as shown in Fig. 11 [15]. The rate of growth of the apatite layer increases with increasing temperature of the second treatment, giving 1.7 and 7.0 μm per day in 1.5 SBF at 36.5 and 60°C, respectively. The activation energy for the growth, which was obtained from an Arrhenius plot of the growth rate against reciprocal absolute temperature, is 43.2 kJ mol^{-1}. This value is much higher than the activation energy for ion diffusion in aqueous solution. The rate of growth of the apatite layer also increases with increasing ion concentrations of the second treatment at a constant temperature, as shown in Fig. 12 [15]. A plot of the growth rate of the apatite layer against the relative supersaturation of the solution on a logarithmic scale gives a slope of 1.3. When the

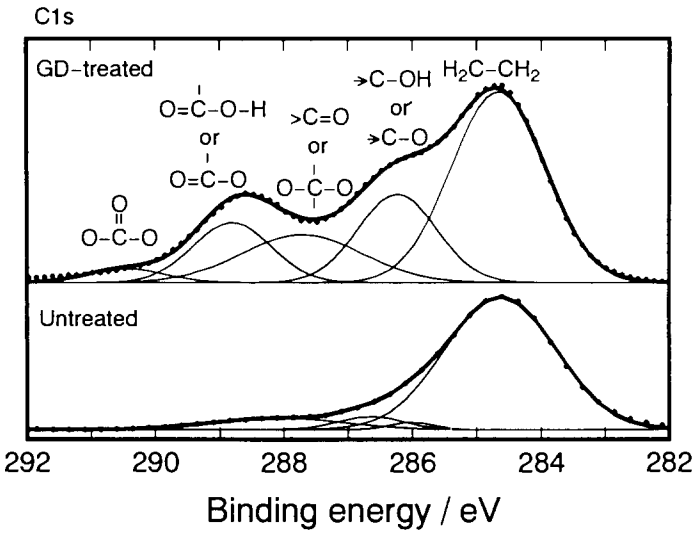

Fig. 10. C_{1S}-XPS spectra of PET substrates treated and untreated with glow discharge for 30 s.

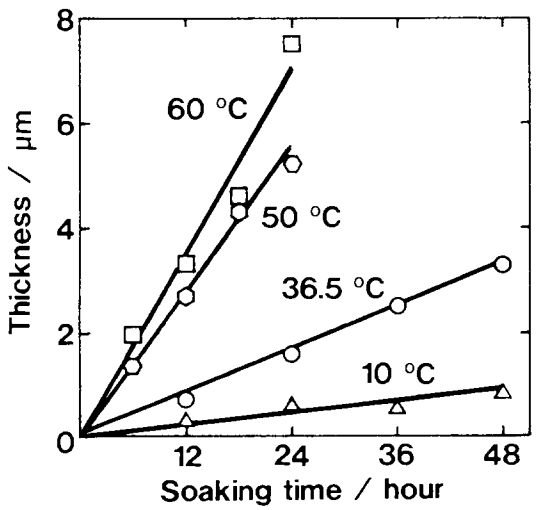

Fig. 11. Growth of apatite layer in 1.5 SBF at various temperatures.

solution for the second treatment is shaken at a rate of 120 strokes min^{-1}, the rate of growth of the apatite layer becomes twice that under static conditions. These results indicate that the growth of the apatite layer is controlled by the transport of the ion across the interface between the crystal and the fluid.

The apatite formed by the present process is represented by the general formula $Ca_{10-x}[(CO_3, HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}]$. As the ion concentrations of the solution

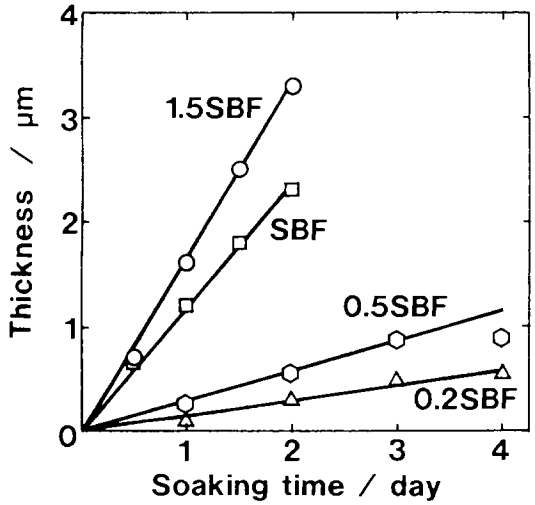

Fig. 12. Growth of apatite layer in solutions with different ion concentrations at 36.5°C.

for the second treatment increase from 0.75 to 2.0 times those of SBF, a larger amount of HPO_4^{2-} substitutes for PO_4^{3-} in the apatite and, accordingly, the Ca^{2+} ion defect increases. Consequently, the Ca/P atomic ratio in the apatite decreases from 1.52 to 1.36. The Ca/P ratio in the apatite formed in SBF was 1.47 whereas that of the apatite in natural bone is reported to be 1.57–1.62. When the HCO_3^- ion concentration in SBF is increased up to the value of 27.0 mM, equal to that in human blood plasma, by increasing the CO_2 gas pressure in the atmosphere, the CO_3^{2-} ion rather than the HPO_4^{2-} ion substitutes for the PO_4^{3-} ion in the apatite, and, accordingly, the P concentration decreases. Consequently, the Ca/P atomic ratio of the apatite becomes 1.57, almost equal to that in bone apatite.

The adhesive strength of the apatite layer formed by the present process to the substrate varied considerably with the kind of polymer, as shown in Table 1. With the glow discharge pretreatment in O_2 gas, it increased remarkably up to 10 MPa at

Table 1
Adhesive strength of apatite layer on polymers untreated and treated with glow discharge

Polymer	Adhesive strength/MPa	
	Untreated	GD-treated
PET	3.48	9.77
PMMA	1.06	5.76
PESF	4.40	9.55
Nylon 6	0.60	7.03
PE	1.93	7.51
PTFF	$< 1.10 \times 10^{-2}$	2.14

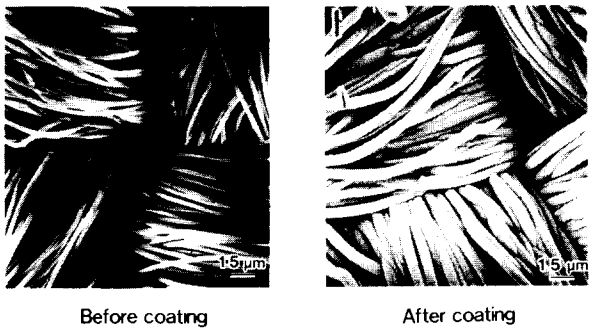

Fig. 13. SEM photographs of PET fine fiber fabric before (left) and after (right) coating of apatite.

maximum, as shown in Table 1 [14]. This might be attributed to the formation of a bond between hydroxyapatite and the polar groups formed on the polymers by the glow discharge.

The bone-like apatite layer can be formed uniformly not only on a flat surface but also on the curved surfaces of fine fibers constituting a fabric, as shown in Fig. 13 [13]. This fabric can be bent sharply without peeling off the apatite layer. If this kind of apatite–organic-polymer composite can be fabricated into a three-dimensional structure analogous to natural bone, the resultant composite is expected to exhibit analogous mechanical properties to those of natural bone as well as high bioactivity, and hence have great potential as bone-repairing materials.

Acknowledgements

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