DEVELOPMENT OF ELECTROCHEMICAL APATITE-COATING ON TITANIUM FOR BIOLOGICAL APPLICATION

Seiji Ban*
(*Corresponding author: sban@denta.hal.kagoshima-u.ac.jp)
Kagoshima University Graduate School of Medical and Dental Sciences
8-35-1, Sakuragaoka, Kagoshima 890-8544, Japan

Keywords: Electrochemistry, Apatite, Biomaterial

Needle-like apatites were successfully formed on titanium plate, bar, and mesh by a hydrothermal-electrochemical method below 200°C in an electrolyte containing calcium and phosphate ions. The deposited needle-like apatites were homogeneously and vertically oriented to the substrate surface. Morphology and crystallinity of the deposited apatite were easily regulated by the electrolytic conditions such as composition and temperature of electrolyte, current density, and substrate. It was confirmed that the electrochemical deposition of apatite had great effect on the promotion of the bioactivity of implant. Furthermore, we applied to produce a novel fabrication method of a composite membrane consisting of oriented needle-like apatite and biodegradable polymer for tissue regeneration in dentistry and medicine.

(Received June 30, 2004; Accepted September, 2, 2004)

INTRODUCTION

Calcium phosphate coating of metallic prostheses has received recently, because these coatings have apparent effect to accelerate bone fixation during the early stages after implantation. There has been a considerable work on these calcium phosphate coatings and many coating methods for them have been reported: e.g., plasma spraying, sputtering, HA-containing glass coating, hot-pressing, Laser beam, ion-plating, ion-beam dynamic-mixing, Ca-ion implantation, sol-gel, and electrochemical method, etc. Electrochemical method is particularly attractive for coating on samples having irregularly shaped substrate. Two major groups for electrochemical deposition of calcium phosphate have been reported. One of these methods is electrophoretic technique. Redepenning and Mclsaac reported that brushite (CaHPO₄·2H₂O, referred to DCPD) was formed on 316L stainless steel plate or titanium mesh at room temperature from aqueous solutions saturated with Ca(H₂PO₄)₂. Shirkhanzadeh demonstrated that thin carbonate apatite coating films on Ti-6Al-4V plate were obtained by using electrochemical technique in aqueous solution which was prepared by dissolution of hydroxyapatite [Ca₁₀(PO₄)₆(OH)₂, referred to HA] into 1 M NaCl solution adjusted to pH 4.4 by the addition of hydrochloric acid. He also reported that periodic pulse of potential leads to obtain uniformly deposited coatings on porous titanium substrate in acidic mixed solution of CaCl₂ and NH₄H₂PO₄. Royer and Rey reported that the calcium phosphate film precipitated on the titanium cathode in the solution saturated with brushite and added of KNO₃ and NaHCO₃. Monma reported that calcium-deficient apatite was deposited by electrochemical method on stainless steel plate in the mixture solution of Ca(H₂PO₄)₂·H₂O and NaNO₃ and/or NaF. Okido et al. investigated the influence of hydrogen peroxide on the electrochemical deposition of apatite on titanium. Manso et al. applied electrochemical HA coating to positive electrode in basic conditions. Hu et al. reported that vinyl acetate was added in the electrolyte to improve the adhesion between deposited HA and titanium.

In recent years, we have been working on electro-chemical deposition of apatite in a simulated body fluid (SBF) and a modified SBF. This review describes advances in synthesis of biological apatite by the electrochemical deposition in aqueous systems and its deposition process, on the basis of our previous reports.

CALCIUM ORTHOPHOSPHATE COMPOUNDS AND HYDROLYSIS REACTION

Because of its crystallographical similarity to various calcified tissues of vertebrates, HA has attracted much attention as a substitute material for damaged teeth or bone over the past several decades. Well-known calcium orthophosphates other than HA are tricalcium phosphate [Ca₃(PO₄)₂, referred to TCP],
tetracalcium phosphate [Ca₄(PO₄)₂·5H₂O, referred to TTCP], octacalcium phosphate [Ca₈H₂(PO₄)₆·5H₂O, referred to OCP] and amorphous calcium phosphate (referred to AP). Orthophosphate salts, which have PO₄³⁻ groups, are distinguished from metaphosphates and pyrophosphates, which have PO₃²⁻ and P₂O₇⁴⁻ groups, respectively. The principal calcium ortho-phosphate compounds in the order of Ca/P molar ratio are also the order of increasing basicity. With the exception of TTCP, all non-apatitic calcium phosphate salts have Ca/P ratios lower than that of HA. As a result, H₃PO₄ would be released into the solution as a byproduct of the hydrolysis reaction.

Although HA is the thermodynamically most stable calcium phosphate phase under physiological conditions, it is now generally accepted that during precipitation process, other phases such as DCPD and OCP may be involved as precursors. Therefore, the crystal phase of precipitated calcium phosphates in aqueous systems strongly depends on pH of the solution.

ELECTROCHEMICAL DEPOSITION OF APATITES IN AQUEOUS SYSTEMS

Fig.1 shows schematic drawing of electrochemical deposition of calcium phosphates on a cathodic titanium surface. Electrochemical deposition of calcium phosphates is based on the pH-dependent solubility which decreases with increasing pH and increasing temperature. During cathodic polarization of a metal in an aqueous electrolyte, the following reaction occur at the surface of the cathode (reduction of water, proton discharge, reduction of dissolved oxygen):

\[ 2H₂O + 2e^- \rightarrow H₂↑ + 2OH^- \] (1)
\[ 2H₃O^+ + 2e^- \rightarrow H₂↑ + 2H₂O \] (2)
\[ O₂ + H₃O^+ + 4e^- \rightarrow 3OH^- \] (3)

which results in the formation of hydroxyl ions and hence alkalization close to the surface.44

On the other hand, it is known that the solubility of calcium phosphate salts decreased with the solution temperature. Relation between the solubility product, Kₛ, for hydroxyapatite and the solution temperature, T, is given by:

\[ \log Kₛ = - \frac{8219.41}{T} - 1.6657 - 0.098215T \] (4)

According to this equation, the solubility of hydroxyapatite shows the maximum at 16°C.

EFFECT OF INORGANIC CONSTITUENTS IN THE ELECTROLYTE

Fig.2 shows SEM micrographs of the titanium surface before and after loading of -2V for 5 min, 1 hr, and 24 hr at 22°C in SBF.13 The amounts of granular products increased with loading time of the cathodic potential. Table 1 shows the summarized results of X-ray diffraction (XRD), infrared spectroscopy (IR), and energy dispersive X-ray analysis (EDX) of the products formed on the cathodic titanium electrode titanium after loading of -2V for 1 hr at 22°C in eleven kinds of electrolytes.12

The products in No. 1, 6 and 8 solution were amorphous carbonate calcium phosphate containing a small amount of Mg, one in No. 3 solution was amorphous magnesium phosphate, one in No. 4 solution was amorphous carbonate calcium phosphate without magnesium, ones in No. 5 and 7 solutions were Mg(OH)₂, ones in No. 9, 10, and 11 solutions were amorphous carbonate calcium phosphate containing CaCO₃. If cathodic potential is loaded onto the working electrode, H⁺ ions move forward it first, because ionic mobility of H⁺ is larger than those of the other ions.46 Next, not only cations but also some anions move to the cathodic electrode, because the electrostatic effects between anions and cations create some ion atmosphere. Thus, it seems that the diffusion process due to the movement of ions is a rate-determining step in the electrochemical synthesis of calcium phosphate.13

Fig.1 Schema of electrochemical deposition of calcium phosphates on a cathodic titanium surface.

Fig.2 SEM micrographs of the titanium surface before and after loading of -2V for 5 min, 1 hr, and 24 hr at 22°C in SBF.
Development of Electrochemical Apatite-Coating on Titanium for Biological Application

With diffusion, the concentrations of these ions increase around the cathode. Although there are many combinations of cations (H⁺, Na⁺, K⁺, Ca²⁺ and Mg²⁺) and anions (OH⁻, Cl⁻, HCO₃⁻ and HPO₄²⁻) in the electrolytic solution, the solubility of the compound consisting of Ca²⁺, Mg²⁺, HPO₄²⁻ and OH⁻ is quite low among them. Therefore, it seems that the insoluble phosphate of calcium and magnesium easily precipitates at the cathodic site. It can be considered that Mg(OH)₂ is formed after HPO₄²⁻ ions are completely consumed in the reaction with calcium and magnesium over a long period of deposition such as 24 hr.

On the other hand, the value of pH increases due to the discharge of H⁺ ions at the cathodic site. Thus, the increase in the amount of ions and the pH value leads to production of the insoluble phosphates. According to Faraday's law, the amount of product is proportional to the electric charge. However, the total amount of products deposited on the working electrode is extremely smaller than that theoretically calculated from the total electric charge. For instance, the total amount of calcium and magnesium deposited after loading of -2V for 1 hr at 22°C was about 1.8 μmol/cm², while the theoretical amount was 141 μmol/cm². It seems that the discrepancy depends on the electric charge consumed for the formation of gases and heat generation.

During the loading of cathodic potential, bubbles were vigorously formed around both the working and the counter electrodes. Furthermore, the pH value of the solution increased after the experiment. It seems that bubbles around the working electrode are H₂ gas and those around the counter-electrode are Cl₂ gas, as in the case of electrochemical decomposition of NaCl solution, because the predominant ions of the electrolyte used are Na⁺ and Cl⁻. Thus, the electric charge was mainly used for the reduction of H₂O to H₂ gas at the cathode and oxidation of Cl⁻ to Cl₂ gas at the anode. From these results, the deposition processes at cathodic site can be summarized by the several reactions:

\[
\begin{align*}
2\text{H}_2\text{O} + 2e^- & \rightarrow \text{H}_2 + 2\text{OH}^- \quad (5) \\
3\text{Ca}^2^+ + 2\text{HPO}_4^{2-} + 2\text{OH}^- & \rightarrow \text{Ca}_3(\text{PO}_4)_2 \downarrow + 2\text{H}_2\text{O} \quad (6) \\
3\text{Mg}^2^+ + 2\text{HPO}_4^{2-} + 2\text{OH}^- & \rightarrow \text{Mg}_3(\text{PO}_4)_2 \downarrow + 2\text{H}_2\text{O} \quad (7) \\
\text{Mg}^2^+ + 2\text{OH}^- & \rightarrow \text{Mg(OH)}_2 \downarrow \quad (8) \\
2\text{Ca}^2^+ + 2\text{HCO}_3^- & \rightarrow 2\text{CaCO}_3 \uparrow + \text{H}_2\text{O} \quad (9)
\end{align*}
\]

In this way, the deposition process progresses due to the movement of Ca²⁺ and Mg²⁺ ions through the diffusion layer on the cathodic site. Furthermore, Ca₃(PO₄)₂ forms a homogeneous solid solution with Mg₃(PO₄)₂ and reacts with H₂O to form a calcium-deficient HA with low crystallinity, and OH⁻ ions are replaced by carbonate ions. Consequently, the products on the titanium are amorphous calcium phosphates containing a small amount of magnesium in the adequate condition, whereas the products sometimes contain Mg(OH)₂ and/or CaCO₃ in the condition of the solution without or low content of HPO₄²⁻ ions due to the consumption of the electrochemical reaction.

**EFFECT OF PH BUFFER**

Fig.3 shows Ca contents derived from EDX spectra of the titanium electrodes after loading of cathodic potential (-2V) for 1 hr at 22°C in Ca/P varied modified SBF with and without pH buffer. Amounts of Ca might correspond to the amounts of the products consisting of calcium phosphate, because the chemical composition of the products can be assumed to be nearly the same, according to the similarity in the morphology, FTIR spectra, and X-ray diffraction patterns. Furthermore, it was revealed from EDX results that Ca/P ratio of the deposits is independent on the deposited amounts. Amounts of Ca of the specimens obtained in the electrolyte with buffer were more ten times larger than those without buffer. It can be said that the addition of pH buffer into the electrolyte had a great effect on the amounts of the products on the electrode.

**Table 1** Results of XRD, IR, and EDX for the products formed on the titanium after loading of -2V for 1 hr at 22°C in eleven kinds of electrolytes.

<table>
<thead>
<tr>
<th>No</th>
<th>Solution</th>
<th>Crystal phase</th>
<th>Molecular group</th>
<th>Ca/P</th>
<th>Ca/P</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SBF</td>
<td>amorphous</td>
<td>PO₄, CO₃, OH</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>saline</td>
<td>(No product)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>No Ca²⁺</td>
<td>amorphous</td>
<td>PO₄, CO₃, OH</td>
<td>++</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>No Mg²⁺</td>
<td>amorphous</td>
<td>PO₄, CO₃, OH</td>
<td></td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>No K⁺ &amp; HPO₄²⁻</td>
<td>Mg(OH)₂</td>
<td>CO₃, OH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>No HCO₃⁻</td>
<td>amorphous</td>
<td>PO₄, CO₃, OH</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>No HPO₄²⁻</td>
<td>Mg(OH)₂</td>
<td>CO₃, OH</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>8</td>
<td>2P</td>
<td>amorphous</td>
<td>PO₄, CO₃, OH</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>9</td>
<td>2Ca</td>
<td>CaCO₃</td>
<td>PO₄, CO₃, OH</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>10</td>
<td>2Ca²⁺</td>
<td>(CaCO₃)</td>
<td>PO₄, CO₃, OH</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>11</td>
<td>4Ca⁴⁺</td>
<td>CaCO₃</td>
<td>PO₄, CO₃, OH</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Underlined molecular groups showed the strong absorption peaks in the IR-spectrum.

**Fig.4** shows pH change of the electrolyte in the center of the cell during a cathodic potential (-2V) at 22°C in Ca/P=1.5 solution with and without buffer. The pH of the electrolyte without buffer is drastically increased, whereas the pH of the buffered solution is slightly increased. The other Ca/P ratio solutions
showed similar pH change with regard to the loading time. It can be understood that the pH of electrolyte changed with the above mentioned chemical reactions. During current loading, the pH in the electrolyte around the cathode increases with formation of OH⁻. As the pH of the solution without buffer increased in all over the solution by agitation with magnetic stirrer, the precipitation of the calcium phosphate in the solution without buffer was observed in all over the solution as well as on cathode electrode, and the amount of products on the electrode was consequently quite small. On the other hands, the pH of the solution with buffer apart from the electrode changed little except around the electrode due to the inhibition by the buffer reaction. Therefore, the calcium phosphate deposited predominantly on the electrode, because the increasing of pH was localized around the cathode.

This pH distribution around the electrode was confirmed by pH imaging microscopy.\textsuperscript{26}

EFFECT OF ELECTROLYTE TEMPERATURE AND CURRENT DENSITY

Because the electrolyte composition had a great effect on the electrochemical deposition of calcium phosphates in aqueous systems as described above, the Ca/P=1.5 modified SBF solution was employed to all the following experiments. This solution was prepared by dissolving given amounts of reagent-grade chemicals of 137.8 mM of NaCl, 1.67 mM of K₂HPO₄ and 2.5 mM of CaCl₂·2H₂O into distilled water. The solution was buffered to a pH value of 7.2 with 50 mM trishydroxyamino-methane and hydrochloric acid.

Figs.5 and 6 show FE-SEM micrographs of the deposits formed under 1.3 and 12.9 mA/cm² for 1 h in the electrolyte at 4-92°C.\textsuperscript{16,18} The deposits formed under 1.3 mA/cm² have granular and plate-like shape at 4-37°C and 52-92°C, respectively.
Development of Electrochemical Apatite-Coating on Titanium for Biological Application

The thickness and the width of the plate-like deposits increased with the electrolyte temperature. Whereas, the deposits formed under 12.9 mA/cm² had granular and needle-like shape at 4-37°C and 52-92°C, respectively. The length and width of the needle increased with the electrolyte temperature. It is concluded that the morphology of the deposits strongly depends on the electrolyte temperature and the current density.

Fig.7 shows XRD patterns of the deposits formed under 1.3 and 12.9 mA/cm² for 1 h at 52-92°C.

Fig.8 shows FTIR spectra of the deposits formed under 1.3 and 12.9 mA/cm² for 1 h at 52-92°C.

FTIR spectra under 1.3 and 12.9 mA/cm² at 4, 22, and 37°C had single PO₄ absorption peaks around 1030 cm⁻¹ and 580 cm⁻¹, indicating calcium phosphate with quite low crystallinity.⁴⁹ The spectra under 1.3 and 12.9 mA/cm² at 52°C had a strong PO₄ absorption peak at 1030 cm⁻¹ and splitted PO₄ absorption peaks 600 and 580 cm⁻¹, indicating apatite crystal. The spectra under 1.3 mA/cm² at 62-92°C showed split PO₄ peaks due to OCP at 1105, 1075, 1035 and 1025 cm⁻¹,⁵⁰ whereas those under 12.9 mA/cm² at 62-92°C showed the PO₄ absorption peaks due to apatite. Furthermore, all the spectra had the bands due to CO₃ and OH groups at 1430 and 1650, respectively. It was also found that CO₃ band of the deposits formed under 12.9 mA/cm² decreased with increasing temperature of the electrolyte, indicating the carbonate content decreased with it.

From these characterization results of SEM, XRD and FTIR, granular-shape deposits were identified as carbonate-containing amorphous calcium phosphates, plate-like ones as carbonate-containing apatite or a mixture of OCP and apatite, and needle-like ones as carbonate-containing apatite oriented to c-axis direction.

OCP is known to grow two dimensionally on the (100) face, followed by hydrolysis to a two unit thickness of HA, and the process is repeated.⁵¹ Because of the similarity in crystal structure between OCP and HA, it has been proposed that epitaxial intergrowths in the <100> direction of these two materials at the unit cell level may be responsible, in part, for the non stoichiometry of biological calcium-deficient apatites.⁵² It is obvious from platy morphology of OCP crystallites that the growth mechanism for a layer structure such as OCP involves apposition of ions on the end faces and edges to produce blades or ribbons elongated and parallel to c-axis and broadened along b-axis.⁵¹ Then, it is often described that OCP acts as a template by the epitaxial apatite crystal growth in biomineralization process.⁵³ In the hydrolysis of OCP by chemical reaction of dicalcium phosphate dihydrate and calcium carbonate, the crystal phase of the products depended upon the reaction time, reaction temperature, and composition of suspension, and then pure OCP could be obtained in an appropriate combination of these factors.⁵⁴,⁵⁵ In case of hydrolysis of OCP, LeGeros et al.⁵⁶ demonstrated that transformation of OCP to apatite occurred by the process of dissolution and subsequent precipitation. Furthermore, it is frequently reported that plate-like apatite is observed when it is formed in relatively low pH and OCP is also present. Monma et al.⁵⁷ reported that mixture of apatite and OCP crystallized as these blades when aqueous suspensions of α-TCP powder were treated at 80°C and at fixed pH 4.6-4.3, and there was no difference in morphology between apatite and OCP, and the apatite was elongated along the (100).
These results suggest that the plate-like OCP crystals form in the early stages of electrochemical deposition and convert to the plate-like carbonate-containing apatites when the lower current density is loaded at 62-92°C. It seems to be caused by the relatively low pH of the electrolyte around the cathode due to the low current density.

HYDROTHERMAL-ELECTROCHEMICAL DEPOSITION OF APATITE

As mentioned above, needle-like apatite crystals were formed on the electrode by loading of relatively high current density in the electrolyte without magnesium ion at 62-92°C. The crystallinity of apatite in the products increased with the electrolyte temperature. It was also found that the needle-like apatite had a great effect on bonding between the implants and bone at the early stages after implantation. Then, it can be expected that the electrochemical apatite deposition at higher temperature may be more suitable method for the biological applications. Therefore, an electro-chemical synthesis under high pressure, namely hydrothermal-electrochemical synthesis, was employed to form the apatite at more than 100°C. The autoclave contained 1 L of the electrolyte in a Pyrex glass-beaker and was sealed by bolting under a Teflon gasket. The electrolyte was heated in a stainless steel-autoclave assembled with two electrodes, a stirring screw, a pressure gauge, a pressure valve, a thermo-couple and an electric heater (Fig.9). The counter electrode as anode was platinum plate, 20x20x0.5 mm, and the working electrode as cathode was a commercially pure titanium, 20x40x0.5 mm.

The deposited weight gain increased with the electrolyte temperature up to 150°C and slightly decreased above it. The weight gain at 150°C was about 50 mg and about four times larger than that at 80°C, about 12 mg. As mentioned above, the deposited amount increased with the electrolyte temperature in the range from 4 to 92°C. Then, it can be inferred that the deposited amount increases with the electrolyte temperature in the range from 4 to 150°C and decreases above it.

Fig.10 shows FE-SEM micrographs of the deposits formed at 100, 150, and 200°C. All the deposits are needle-like shape having a defined hexagonal crystal habit. The end of the hexagonal bar was flat below 150°C, step at 170°C, and screw at 200°C. Furthermore, it was found that the long needle-like crystals grew up perpendicular to the substrate. Morphology and oriented growth of these deposits were similar to those prepared by a thermal dissociation.

Fig.11 shows the longitudinal length of the hexagonal crystals as a function of temperature of the electrolyte. The longitudinal length of the hexagonal crystals increased with the electrolyte temperature: e.g. about 12 and 240 μm at 100 and 200°C, respectively.
The length of one side of the hexagonal crystals also increased with the electrolyte temperature: e.g. about 0.3 and 7 μm at 100 and 200°C, respectively. However, it was difficult to determine the length of the deposit formed at 80°C, because they were not defined hexagonal shape.

The degree of crystallinity of the calcium phosphates, obtained from FTIR spectra, increased with the electrolyte temperature. The value at 200°C was about 90%. On the other hand, the degree of crystallinity at 52°C was about 27%. These results demonstrated that the deposits became to be similar to stoichiometric HA with increasing the electrolyte temperature.

Fig. 12 shows relation between Ca/P ratio of the deposits and the electrolyte temperature. The Ca/P ratio increased with the electrolyte temperature. The Ca/P ratio at 200°C was about 1.65, indicating the similar composition to the stoichiometric HA having 1.67 of Ca/P ratio. Hexagonal bars of HA crystal deposited on titanium electrode using the hydrothermal-electrochemical method under 12.5 mA/cm² for 1 hr at 80-200°C. The deposited amount increased with the electrolyte temperature up to 150°C and slightly decreased above it. It seems to be caused by the synergistic effect of diffusion rate of calcium and phosphate ions, solubility of calcium phosphate and pH of the electrolyte around the cathode. The deposits were identified as HA crystal rods grown along c-axis and perpendicular to the substrate. These characterization results demonstrated that the crystallinity of the deposited HA increased continuously with the electrolyte temperature and closed to stoichiometric HA. At 150 - 160°C, the HA rod grow most homogeneously perpendicular to the substrate and the edge of the needle has a flat hexagonal plane.

**Fig.12 Ca/P ratio of the deposits formed under 12.5 mA/cm² for 1 hr in the electrolyte as a function of the electrolyte temperature.**

EFFECT OF SUBSTRATE MATERIALS

Pure titanium plate has been employed as the substrate in fundamental studies mentioned above. Because there was no remarkable effects of metal substrates (pure gold, platinum, titanium, nickel, iron, copper and zinc, and stainless steel, Ti-6Al-4V, Ni-Ti and Co-Cr alloys) on the microstructure of the deposits formed at 92°C, except pure aluminum plate. However, hydrothermal-electrochemical deposition of apatite, namely apatite formation above 100°C in an electrolyte under high pressure, may increase susceptibility in the corrosion of the substrate metal. It is necessary to further investigate whether the other alloys are durable as substrate for hydrothermal-electrochemical deposition of apatite.

Table 2 shows the summary of the crystal phases of the deposits formed on 9 kinds of metals at 12.5 mA/cm² for 1 hr at 100, 150, and 200°C.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>100°C</th>
<th>150°C</th>
<th>200°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titanium</td>
<td>HA</td>
<td>HA</td>
<td>HA</td>
</tr>
<tr>
<td>Zinc</td>
<td>HA, Parascholzite</td>
<td>Parascholzite, ZnO</td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td>HA, β-TCP</td>
<td>HA, β-TCP</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>HA, β-TCP</td>
<td>HA, β-TCP</td>
<td></td>
</tr>
<tr>
<td>SUS316</td>
<td>HA, β-TCP</td>
<td>HA, β-TCP</td>
<td></td>
</tr>
<tr>
<td>Ti6Al4V</td>
<td>HA</td>
<td>HA</td>
<td>HA</td>
</tr>
<tr>
<td>TiNbTaZr</td>
<td>HA</td>
<td>HA</td>
<td>HA</td>
</tr>
<tr>
<td>AgIn</td>
<td>HA</td>
<td>HA</td>
<td>HA, β-TCP</td>
</tr>
<tr>
<td>12%AuPdAg</td>
<td>HA, β-TCP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2 Crystal phases of the deposits formed on 9 kinds of metals at 12.5 mA/cm² for 1 hr at 100, 150, and 200°C.**

![Fig.13 Shear bonding strengths of the deposits, formed at 12.5 mA/cm² for 1 hr at 100, 150, and 200°C, to 6 kinds of substrate metal.](image)

**Fig.13 Shear bonding strengths of the deposits, formed at 12.5 mA/cm² for 1 hr at 100, 150, and 200°C, to 6 kinds of substrate metal.**
9 kinds of metal. At 150°C, the deposits on pure iron and stainless steel plates were mixtures of HA and β-TCP, that on pure zinc was a mixture of HA and parasholzite, CaZn$_2$(PO$_4$)$_2$·2H$_2$O, and the deposits on the other metals were only HA. These results demonstrated that only the deposits up to 200°C on pure titanium, Ti6Al4V and TiNbTaZr were a single phase of HA and the others were mixtures of two crystal phases. It was impossible to calculate the degree of the deposits at 150°C and 200°C on pure iron, because it was no clear absorption peaks of PO$_4$ in these spectra due to the formation of parasholzite, as shown in Table 2. The crystallinity of the deposits on the other metals increased with the electrolyte temperature except those on pure nickel, pure iron, and AgIn. The crystallinity of the deposits at 200°C on pure nickel, pure iron, and AgIn was smaller than those at 150°C.

Fig. 13 shows shear bonding strengths of the deposits, formed at 100, 150 and 200°C under 12.5 mA/cm$^2$ for 1 hr, to 6 kinds of substrate metal. The bonding strengths of the deposits to each substrate metal increased with the electrolyte temperature at which the deposits were formed. However, there were no significant differences in the bonding strengths of the deposits to each metal, although the bonding strengths of the deposits to each substrate metal increased with the electrolyte temperature. These weak bonding strengths were nearly the same as those of polycarboxylate and glassionomer cements to electroplated-tinned gold. Furthermore, all fractures occurred at interface between the substrate and the deposits. Therefore, it seems that the bonding of the deposits to the substrate is due to the second chemical bond such as Van der Waals force, not to the first chemical bond such as ionic bond and covalent bond, etc. It also seems that fine needle shape of the deposits is one of the reasons of the weak bonding. The modification using super-critical water improved the mechanical strength of the deposited apatite, but worsened bio-compatibility. However, the surface of the implant usually was not smooth due to sandblasting with titanium particles and alumina powder. Then, the deposits in the concave part are untouchable during manipulation such as sterilization and surgical operation. Furthermore, the primary purpose of the surface modification with apatite coating on metal implant is to make bio-compatible base for new bone formation and the artificial preexisted apatite finally converts to biological apatite as new bone. Therefore, the weakness of bonding strength is not a fatal problem for the surface modification of implant. The requirements of substrate for electrochemical deposition of apatite are to have adequate electro-conductivity and excellent biocompatibility, and not to contaminate the deposits.

### BIOCOMPATIBILITY OF THE DEPOSITED APATITE

Morphology of the deposited apatite is one of the most important factors on the biocompatibility in the living tissue. Then, the next study was focused on systematic investigation of the effect of the electrolyte temperature, the current density, and the current loading time on both size and shape of the deposited apatite on titanium plate in an electrolyte.

The electrolyte temperature was maintained at 90-200°C under 12.5 mA/cm$^2$ for 60 min. The current density was maintained at 5.0, 8.8, 12.5, 18.8 and 25.0 mA/cm$^2$ for 60 min at 100, 150 and 200°C. The current loading period of time, it was varied for 10-120 min under 12.5 mA/cm$^2$ at 100, 150 and 200°C. After loading of a constant current in these 45 kinds of condition, the titanium-cathodes were rinsed with distilled water and dried at 37°C in air. From FE-SEM images of the surface and the section of the deposits, the longitudinal length and the length of one side of the hexagonal apatite needles, respectively corresponding to the length of c- and a-axes, were determined and aspect ratios of c/a were calculated from them. Multivariate analysis was employed to analyze relations between above three electrolytic conditions and dimensional parameters using commercial statistic software.

From these analytic results, the regression equations for the longitudinal length of apatite (c) in μm, length of one side of hexagonal apatite (a) in μm, and aspect ratio of c/a of the needle-like apatite were obtained as follows.

\[
c = 1.3567T + 4.907D + 1.004P - 249.8 (r=0.926) \quad (10)
\]

\[
a = 0.0687T + 0.124D + 0.052P - 11.6 (r=0.903) \quad (11)
\]

\[
c/a = -0.153T + 0.785D - 0.091P + 34.7 (r=0.688) \quad (12)
\]

where $T$ is electrolyte temperature in °C, $D$ is current density in mA/cm$^2$, and $P$ is current loading period of time in min. The analytical results suggest that the morphology of the apatite can be regulated accurately by systematic control of the electrolyte temperature, the current density, and the current loading time. These results revealed that hydrothermal electrochemical deposition of apatite consists of two processes: nucleation and crystal growth, which strongly depend on the electrolyte temperature and current density.

### MORPHOLOGICAL REGULATION OF THE DEPOSITED APATITE

As mentioned above, the weakness of bonding strength is not a fatal problem for the surface modification of implant. It can be confirmed that the implant with this electrochemical apatite coating
showed the higher bonding strengths to bone than those without it\textsuperscript{14,20,21} and were independent on the electrolyte temperature (100, 150, and 200°C) at which the deposits were formed.\textsuperscript{28,30}

Titanium bars with and without the apatite were implanted into the femora of Japanese white rabbit. The rabbits were sacrificed at 3 weeks after implantation and the femora were taken out from the bodies. Immediately, the femora were used for radiographic observation, and the bonding strengths between these specimens and the bone were determined by the pull-out method using Instron testing machine. Fig.14 shows the pull-out bonding strengths between the titanium bars with and without the hydrothermal-electrochemically deposited apatite at various electrolyte temperatures and rabbit femora after 3-week implantation in comparison with our previous results.\textsuperscript{20,21,30} There were no significant differences in the pull-out bonding strengths of the titanium bars coated at 100, 150, and 200°C to the rabbit femora.

Fig.15 shows FE-SEM of the surface of the titanium bar with and without hydrothermal-electrochemically deposited apatite at 100, 150, and 200°C after 3-week implantation in rabbit femora.\textsuperscript{30} All the hydrothermal-electrochemically deposited apatite showed an excellent biocompatibility. The new bone grew into the space between the needle-like apatites. The space of the apatites deposited at 100°C was completely filled with the new bone, whereas that at 200°C was not yet filled with it.

It is concluded that the morphology and chemical properties of the electro-chemically deposited apatite were different in the electrolyte temperature and the apatite at 100°C showed the best bioactivity \textit{in vitro}, because of the solubility and morphology,\textsuperscript{35} whereas the thickness of the apatite coating was the more effective on the bioactivity \textit{in vivo}.\textsuperscript{30}

---

**ELECTROCHEMICAL APATITE COATING ON TITANIUM MESH**

Titanium mesh has been applied to various medical and dental treatments, especially bone regeneration.\textsuperscript{60-63} Because titanium mesh has excellent mechanical properties in terms of stiffness and elasticity, indicating easy to handle during surgery. Furthermore, the titanium mesh can be provided with calcium phosphate coatings. Jansen et al. reported that the magnetron sputtering was employed to prepare the calcium-phosphate-coated titanium mesh having excellent biocompatibility.\textsuperscript{64,65} However, a disadvantage of the magnetron sputtering is that it is difficult to prepare the coating film with homogeneous thickness on a substrate like a mesh having a complicated shape. Then, the hydrothermal-electrochemical apatite coating was applied to improve the bioactivity of titanium mesh.

Fig.16 shows SEM micrographs of the #80 titanium meshes before and after the apatite coating using the hydrothermal-electrochemical method at 100, 150, and 200°C under 12 mA/cm\textsuperscript{2} for 60 min. The deposits were homogeneously formed on the surface of the titanium woven fiber. Needle-like and plate-like deposits were formed on the titanium mesh at 100°C, whereas needle-like deposits were only formed at 150 and 200°C. The thickness of the thin plates at 100°C was about 0.1 μm and the widths were 30-80 μm. The needle-like deposits were 0.5-1 μm in width and about 7 μm in length at 100°C, 2-8 μm in width and 50-70 μm in length at 150°C, and 4-14 μm in width and 60-80 μm in length at 200°C. It is confirmed that the size of the deposits increased with the electrolyte temperature. Furthermore, most of the needles grow perpendicular to the surface of the mesh fiber, and their sharp edges were hexagonal. Fig.17 shows SEM micrographs of the #200 mesh after the apatite coating at 100°C under 50 mA/cm\textsuperscript{2} for 30 min. Fine apatite needles, 0.1-0.3 μm in width and 2-3 μm in length, were formed on the mesh grid surface, and much amount of needles were formed on the wide rim providing a stiffness of the mesh grid. Although the much higher current density was loaded, the apatites on the grids were smaller than those on #80 mesh and plate.
Apatites were homogeneously formed and vertically oriented to the surface of the titanium woven fiber and grid. Both the morphology and dimensions of the deposited apatites could be regulated by the electrolytic conditions. It indicates that the apatite coating by the hydrothermal-electrochemical method is suited to substrate like mesh having a complicated shape and a large specific area. Furthermore, it is attractive that the mesh with the electrochemical apatite coating showed excellent biocompatibility in vitro. It was also reported that the mesh infiltrated with biodegradable polymer also showed good bioactivity in vitro.

PREPARATION OF COMPOSITE MEMBRANE

Guided Tissue Regeneration (GTR) and Guided Bone Regeneration (GBR) are techniques for the treatment of bone defects such as periodontal disease and apical lesion. Physical barriers in the form of a membrane have been placed between the mucogingival flap and bone and tooth/implant surfaces during surgery. The biodegradable membranes are successfully applied to these treatments. Synthetic polymers such as poly-lactic acid, poly-glycolic acid, and poly-lactic/poly-glycolic acid copolymer (PLGA) are frequently used as biodegradable membrane in dental and medical applications. These polymers are absorbed after regeneration of the target tissue. The reasons for using biodegradable membranes are the elimination of a second stage operation, their low rate of exposure, and the high probability of healing with a complete restitution, ad integrum even after an exposure. The PLGA has been used as biodegradable sutures, hemostatic materials, bone fixation materials and guided tissue regeneration (GTR) materials for dental and medical fields. It has been reported that the composite membranes consisting of apatite particles and biodegradable polymer were prepared to improve their mechanical strength and decomposition rate. Then, we developed a novel fabrication method of a composite membrane consisting of oriented needle-like apatite, formed by the hydrothermal-electrochemical method, and biodegradable polymer.

Fig. 18 shows a schematic drawing of the preparation method. 100 mg of PLGA was dissolved in 1.5 ml of dichloromethane (CH₂Cl₂) and was stirred for 3 minutes in the glass beaker. This copolymer solution was dropped on the pure titanium plate coated with the oriented needle-like apatites.
Development of Electrochemical Apatite-Coating on Titanium for Biological Application

using the hydrothermal-electrochemical method. After drying at 37°C for 24 hours, the film was hardened and forced to delaminate by a surgical knife. The size of the membrane was 20 x 40 mm and its thickness was in the range between 50 and 80 μm. The composite membrane appeared to be semitransparent and had many pores, less than 1 mm, due to the evaporation of dichloromethane used as solvent. It was found that the apatite needles were well incorporated in the semitransparent copolymer film. The composite membrane was flexible and able to cut using a conventional scissors to an adequate size. The tensile strength of the composite membrane was similar to that of the original PLGA sheet. Fig.19 shows SEM micro-graphs of the section of the composite membrane. It was found that the apatite needles were well embedded in the copolymer and the tips of the needles were exposed on the one side of the composite membrane in which was contacted to the substrate.

This preparation method of the biodegradable membrane containing oriented needle-like apatite is an application of our previous studies on the hydrothermal-electrochemical deposition of apatite. It is a quite simple and easy method for producing a large sheet of membrane. The properties of the composite membrane can be easily controlled; e.g. the degradation rate of this membrane can be regulated by the thickness of the copolymer, which can be controlled by concentration and dropped volume. The rate of degradation of biodegradable membrane is critical to the success of procedure. Then, it can be expected that the composite membrane consisting of the oriented needle-like apatite and the biodegradable copolymer may have excellent biocompatibility with the surrounding living tissue. We reported that this composite membrane showed a good biocompatibility with soft tissue and hard tissue. Furthermore, BMP was applied on this composite membrane and produced the biologically active membrane.

ASPECTS OF FUTURE RESEARCH

Among various apatite-coating methods, electro-chemical methods have some advantages for biological use: i.e. it can make easily homogeneous coating on the substrates having complicated shape such as dental implant and requires simple and small devices. It was confirmed that the electrochemical deposition of apatite had great effect on the promotion of the bioactivity of implant. The morphology and crystallinity of the apatite were easily regulated by varying the electrochemical condition; e.g. electrolyte composition and electrolyte temperature, current density, and loading time, etc. By applying these advantages of the hydrothermal-electrochemical apatite coating, it can be expected that various novel composite materials will be developed. The future research in this area will be focused on applications to scaffold materials for tissue regeneration.

REFERENCES