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Bioactive Apatite Coating on Titanium Using an Alternate Soaking Process

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We developed a novel apatite coating method that consisted of two-step of chemical treatment: a combined pretreatment of concentrated acid etching and alkaline treatment, followed by alternate soaking. In this study, the effects of the number of reaction cycles, solution temperature, and soaking time on apatite deposition on titanium surface using alternate soaking were investigated. Results revealed that the deposited amount of apatite mainly depended on the number of reaction cycles, and was independent of solution temperature and soaking time. Characterization results revealed that apatite formation using alternate soaking basically depended on ion exchange and adsorption on the pretreated surface. Further, apatite coating using alternate soaking on a 200-grid titanium mesh confirmed that this coating method was applicable for substrates with complicated shapes.

Keywords: Apatite, Titanium, Alternate soaking

INTRODUCTION

Titanium and its alloy have excellent corrosion resistance, mechanical strength, and biocompatibility, and are widely used as implant materials subjected to biomechanical loading[1-3]. It is known that the surface condition of titanium significantly influences its biocompatibility[3]. A lot of techniques have been reported to improve the titanium surface for biological applications using physical and chemical treatments: sandblasting[3], acid etching[3-5], alkaline treatment[6,7], glow discharge treatment[3], and apatite coating. Among these techniques, apatite coating is known to promote direct contact with bone tissue[8-10].

Practically, apatite coating has been widely used in orthopedics and dental materials in order to improve the success rate of bone bonding as well as enhancing tissue compatibility with titanium and its alloys[1,9-11]. For apatite coating, a variety of methods have been reported: plasma spraying[12], ion beam assisted deposition[13], magnetron sputtering[14], sol-gel processes[15], composite glass coating[16], electrochemical deposition[17-20], and alkaline treatment[21]. In particular, chemical treatments such as electrochemical deposition and alkaline treatment are simple and inexpensive methods, because they do not need expensive and large equipment and they allow for homogeneous coating on substrates with complicated shapes.

Previously, we reported on a simple two-step chemical treatment of commercially pure titanium (cpTi) plates[4]. It entailed etching in a concentrated acid followed by alkaline treatment. The result was encouraging in that apatite-forming ability was greatly enhanced on the surface of titanium in a simulated body fluid. It was thus perceived that concentrated H_2SO_4 etching could enhance the apatite-forming ability of alkaline-treated titanium due to the formation of a large amount of sodium titanate with increasing surface area and the formation of TiH_2. It was also reported that apatite coating was possible on hydrophilic polymer-grafted polyethylene films and agarose by an alternate soaking process, whereby the characteristics of the obtained apatite were quite similar to real bone[10,22]. This process is based on the widely known wet process for hydroxyapatite preparation by alternately soaking in $CaCl_2$ /Tris-HCl and Na_2HPO_4 aqueous solutions. However, there were no reports on apatite coating on titanium using the alternate soaking process. It seems that untreated titanium is relatively hydrophobic, and that it is difficult to adsorb Ca and PO_4 ions.

Recently, we developed a novel apatite coating method for titanium, whereby the alternate soaking process was preceded by a combined pretreatment of acid etching and alkaline treatment[23]. It was confirmed that the number of reaction cycles affected the deposited amount of apatite on cpTi plates[23]. Moreover, vacuum firing of the substrate decreased the deposited apatite, although vacuum firing erased the TiH_2 formed during acid etching[24].

In the present study, effects of the number of reaction cycle, soaking time, and solution temperature on apatite deposition on cpTi plates using the alternate soaking process were systematically investigated to determine an optimal coating condition. Furthermore, coating on cpTi mesh was also examined to evaluate the coating ability on substrates with complicated shapes. Finally, the mechanism of apatite coating using alternate soaking was also discussed.

MATERIALS AND METHODS

Pretreatment

Four kinds of pretreatment before apatite coating were undertaken as follows. CpTi plates (Kobelco KS-40, JIS-1, 20×15×0.5 mm) were ultrasonically cleaned in acetone and distilled water for 10 minutes respectively, and dried in air at 37℃ (AS). After drying, the cpTi plates were etched in 48% H_2SO_4 at 60℃ for 60 minutes. After etching, the metal plates were ultrasonically cleaned with distilled water and dried in air at 37℃ (AE). Subsequently, cpTi plates with and without acid etching were soaked in 5 M NaOH at 60℃ for 24 hours (AA and AT). After the alkaline treatment, the metal plates were washed with distilled water and dried in air at 37℃.

Apatite coating on Ti plate by alternate soaking method

Fig. 1 shows the sequence of the alternate soaking cycle. The four kinds of cpTi plate with and without pretreatment were soaked in 0.5 M $CaCl_2$ solution. After taking out from the solution, the cpTi plates were washed with distilled water, whereby remaining moisture on the surface was absorbed using absorbent paper towel and dried with an electric dryer. Subsequently, the specimens were soaked in 0.1 M Na_2HPO_4 solution. After taking out from the solution, cleaning and drying were again undertaken in the same manner. Soaking times in both solutions were 1, 10, and 30 minutes. Temperature of the solutions was maintained at 4, 37, and 60℃. These procedures were repeated sequentially according to the required number of cycles. After 10, 30, and 50 reaction cycles, the cpTi plates were thoroughly rinsed with distilled water and dried in air at 37℃.

Characterization

Surfaces of the specimens were characterized using an X-ray diffractometer (XRD) (RINT-2500, Rigaku, Tokyo, Japan), a Fourier transform infrared reflection microspectrometer (FT-IR) (FT/IR-460 plus, JASCO, Tokyo, Japan), and a scanning electron microscope (SEM) (JSM-5510LV, JEOL, Tokyo, Japan). Ca/P molar ratios of the deposits were analyzed by energy dispersive X-ray spectroscopy (EDX) (JED-2201, JEOL, Tokyo, Japan). Based on the calibration line derived from the measured Ca/P ratio of stoichiometric hydroxyapatite and tricalcium phosphate (TCP), their theoretical values were 1.67 and 1.5. Stoichiometric hydroxyapatite was synthesized by a wet method using H_3PO_4 and $Ca(OH)_2$, fired at 1200℃ and identified by XRD. TCP was a commercial regent-grade chemical. Furthermore, to quantitatively evaluate the deposit amounts, the sum of the atomic fractions of Ca and P was derived from the EDX semiquantitative results. Measurements were repeated four times for each condition and analyzed by Student's *t*-test for multiple comparisons at p=0.05 level.

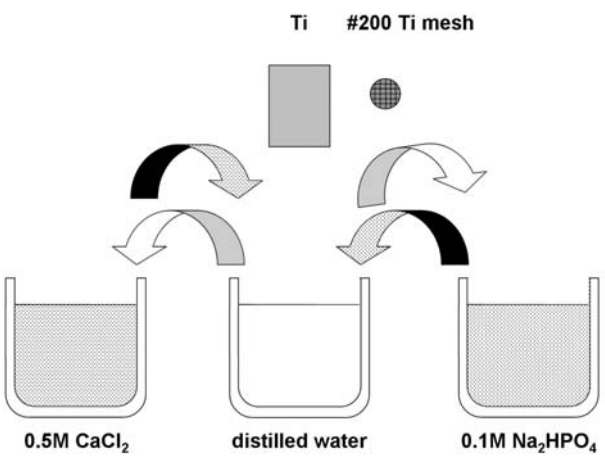

Fig. 1 Schematic drawing of the alternate soaking process.

Coating on Ti mesh

Ti mesh of 200-grid (ø3.05 mm, 25 μm thickness; Okenshoji Co. Ltd., Tokyo, Japan) was treated with four kinds of pretreatment in the same manner as that for the cpTi plates. For mesh pretreatment, etching time in H_2SO_4 was decreased to 30 minutes. This was because 60 minutes of mesh etching could result in over-etching, thereby causing the shape of the mesh to be distorted. In the alternate soaking process, there was only one reaction cycle. Solution temperatures were 20 and 60℃, and soaking time was one minute. After alternate soaking, deposits on cpTi mesh were characterized using SEM and transmission electron microscope (TEM) (JEM-2000EX, JEOL, Tokyo, Japan).

RESULTS

Pretreatment of pure titanium plate for alternate soaking

Fig. 2 shows the SEM micrographs of the surfaces of AS, AE, AT, and AA. AS which was the as-received plate showed a smooth surface. AE showed a macroporous structure consisting of homogeneous micro pits, about 1 μm in diameter, formed by acid etching. AT showed a microporous network structure formed by the alkaline treatment. AA, subjected to a combined treatment of acid etching and alkaline treatment, had a microporous network formed on the macroporous surface.

Fig. 3 shows the XRD patterns of AS, AE, AT, and AA after 10 reaction cycles at 60℃ for 30 minutes. The diffraction peaks assigned to apatite were observed on both AT and AA, but not on AS and AE. Furthermore, the diffraction peaks assigned to apatite of AA were of much larger intensity than those of AT. These results were similar to those at

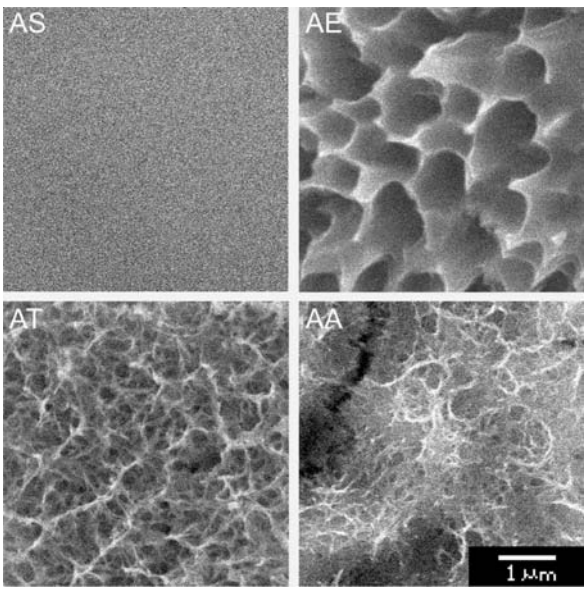

Fig. 2 SEM micrographs of the surfaces of cpTi plates with and without chemical treatment (AS, AE, AT, and AA).

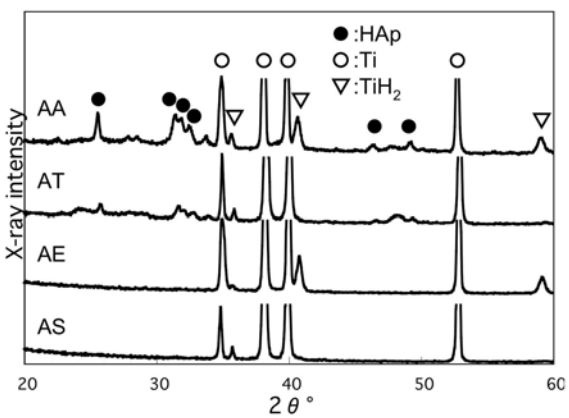

Fig. 3 XRD patterns of cpTi plates with and without chemical treatment (AS, AE, AT, and AA) after alternate soaking for 10 reaction cycles at 60℃ for 30 minutes.

4 and 37℃ for 30 minutes, although the crystallinity of apatite at 4℃ and 37℃ was low. According to our previous results[23], TiH_2 of AE and AA were formed by etching in H_2SO_4.

Fig. 4 shows the SEM micrographs of the surface of AA before and after 50 reaction cycles at 60℃ for one minute. As mentioned above, a microporous network was formed on the macroporous surface of AA. After alternate soaking, the microporous network structure disappeared and unclear-cut deposits were formed homogeneously on the surface.

Apatite formation on pure titanium plate under various conditions of alternate soaking

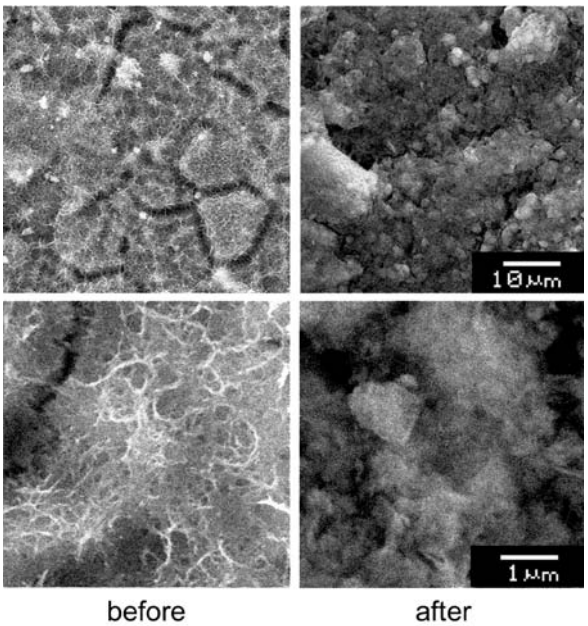

Fig. 4 SEM micrographs of the surface of AA before and after 50 reaction cycles at 60℃ for 1 minute. Lower photo is a higher magnification of the center part of upper one.

Fig. 5 shows the XRD patterns of AA after alternate soaking under the various conditions. Fig. 5(a) shows the XRD patterns of AA after different reaction cycles (10, 30, and 50 cycles) at 60℃ for one minute. Fig. 5(b) shows the XRD patterns of AA after 10 reaction cycles at different solution temperatures (4, 37, and 60℃) for 30 minutes. Fig. 5(c) shows the XRD the patterns of AA after 10 reaction cycles at 60℃ for different soaking times (1, 10, and 30 minutes). The diffraction peak of apatite around 26° and 32° increased with solution temperature and the number of reaction cycles, but no remarkable changes were observed with soaking time.

Fig. 6 shows the EDX spectra of AA before and after 50 reaction cycles at 60℃ for one minute. Before alternate soaking, Ti and O were clearly detected. Although sodium titanate was formed on the surface, Na was not detected. However, this might probably depend on the detectable limitation of the EDX device. After alternate soaking, O, P, Ca, and Ti were clearly detected. Intensity of the substrate Ti dramatically decreased due to interference from calcium phosphate deposits after alternate soaking.

Fig. 7 shows the Ca/P ratio of AA after alternate soaking under various conditions. Ca/P ratio of the deposits increased with the number of reaction cycles ($p<0.05$). However, there were no significant differences in Ca/P ratio of the deposits among different solution temperatures and different soaking times ($p>0.05$).

Fig. 8 shows the sums of the atomic fractions of

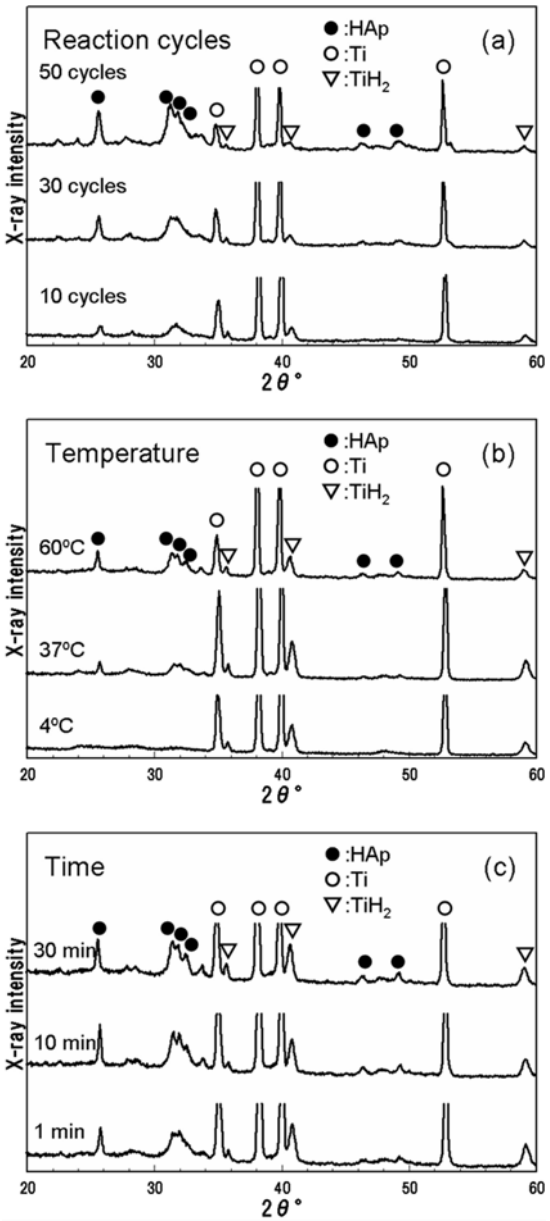

Fig. 5 XRD patterns of AA after 10, 30, and 50 reaction cycles at 60℃ for 1 minute (a), after 10 reaction cycles at 4℃, 37℃ and 60℃ for 30 minutes (b), and after 10 reaction cycles at 60℃ for 1, 10 and 30 minutes (c).

Ca and P derived from the EDX results. The sum of the atomic fractions of Ca and P could be assumed to be indices of the deposited amount of apatite. The sum of the fractions significantly increased with the number of reaction cycles ($p<0.05$), whereas no significant changes were observed with solution temperature ($p>0.05$) and a slight change with soaking time ($p<0.05$), only between 10 and 30 minutes.

Fig. 9 shows the FT-IR reflection spectra of AA after 10, 30, and 50 reaction cycles at 60℃ for one

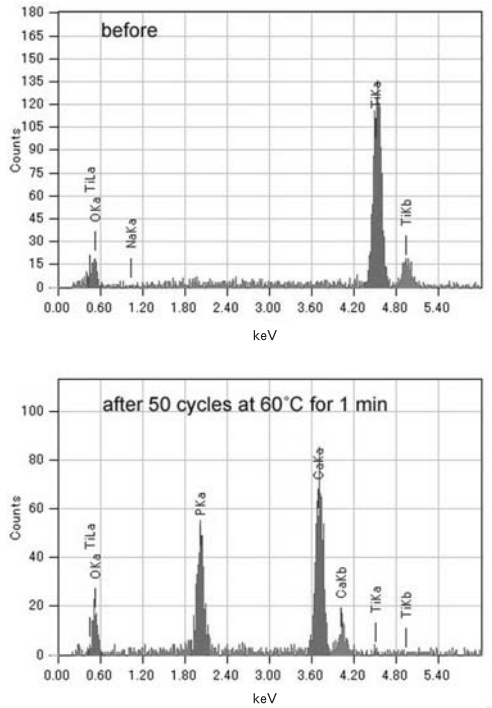

Fig. 6 EDX spectra of AA before and after 50 reaction cycles at 60℃ for 1 minute.

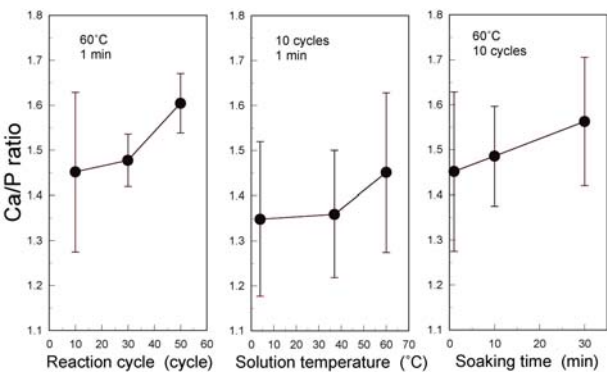

Fig. 7 Change in Ca/P ratio of AA after alternate soaking.

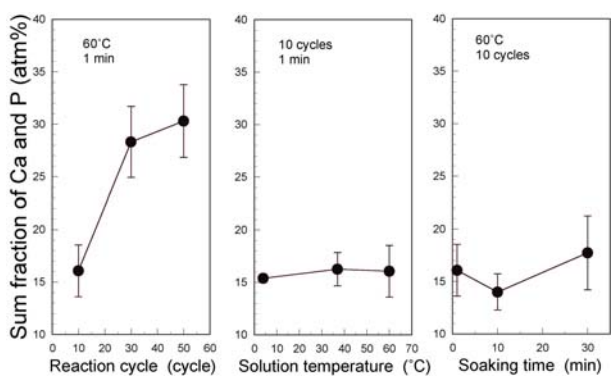

Fig. 8 Change in the sum fraction of Ca and P of AA after alternate soaking.

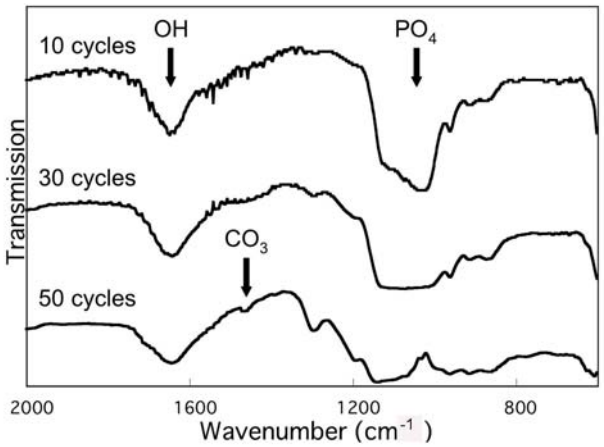

Fig. 9 FT-IR reflection spectra of AA after 10, 30, and 50 reaction cycles at 60℃ for 1 minute.

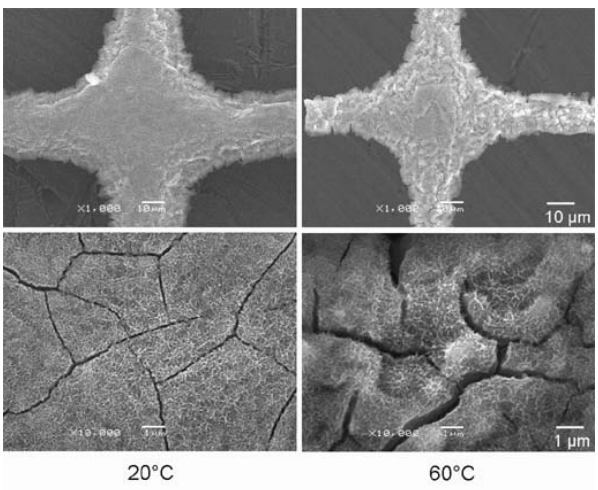

Fig. 10 SEM micrographs of the surfaces of AA mesh after 1 reaction cycle at 20℃ (left) and 60℃ (right) for 1 minute. Lower photo is a higher magnification of the center part of upper one.

minute. The absorption bands around 1000-1150 cm^{-1} assigned to PO_4 of apatite increased with the number of reaction cycles, and the bottom of the peaks of 30 and 50 cycles was saturated due to the large deposited amounts and because of reflection mode. Small peak around 1460 cm^{-1} assigned to CO_3 was only observed in the spectrum after 50 cycles. OH peak around 1650 cm^{-1} was observed in all the spectra.

Apatite formation on pure titanium mesh by alternate soaking

Fig. 10 shows the SEM micrographs of the AA mesh after only one reaction cycle at 20 and 60℃ for one minute. The surfaces of the meshes of AS, AE, AT, and AA before alternate soaking (not shown here) were similar to those of cpTi plate shown in Fig. 2. The surfaces of AA after alternate soaking at 20 and 60℃ were homogeneously covered with the deposits even after only one cycle of alternate soaking. Judging from the crack width, thickness of the deposited layer at 60℃ seemed to be greater than that at 20℃. These results demonstrated that apatite coating using alternate soaking was applicable for substrates with complicated shapes, such as a mesh. Furthermore, a higher soaking temperature produced a larger amount of apatite.

Fig. 11 shows the TEM images and electron diffraction patterns of AS, AE, AT, and AA before and after alternate soaking at 20 and 60℃ of the 200-grid cpTi mesh. Electron diffraction patterns of AS were assigned to α-titanium. The patterns of AE seemed to be due to α-titanium and TiH2. Twisted thin films, perpendicular to the mesh surface, were observed on the surfaces of AT and AA. Judging from these diffraction rings and our previous study[4], the deposits of AT and AA seemed to be sodium titanate with low crystallinity. The length or height of the twisted thin films seemed to be 100-200 nm. It was likely that these twisted thin films constructed the microporous network structure observed in the SEM images (Fig. 2). The length of the needle-like twisted thin films of AA after alternate soaking at 60℃ was shorter than that of AA before soaking, indicating that the twisted thin films were consumed with alternate soaking. Although the TEM image of AA after alternate soaking at 60℃ showed a remarkable change, its electron diffraction rings showed nearly the same rings of AA after alternate soaking at 20℃ assigned to the apatite with a low crystallinity, but different from those before alternate soaking.

DISCUSSION

As shown in Fig. 3, the diffraction peak assigned to hydroxyapatite were observed on both AT and AA, but not on AS and AE after alternate soaking. TiH_2 observed on AE and AA was formed by etching in H_2SO_4. Furthermore, the diffraction peaks assigned to hydroxyapatite of AA were of larger intensity than those of AT. Effects of these pretreatments were similar to those at 4 and 37℃, although the crystallinity of hydroxyapatite at 4 and 37℃ was low. Our previous study revealed that the presence of TiH_2 was independent of the deposited amount[23]. Besides, it was reported that concentrated acid etching enhanced the apatite-forming ability in a simulated body fluid in the same way[4]. Indeed, these results demonstrated that concentrated acid etching enhanced the apatite-forming ability of the alkaline-treated titanium during alternate soaking. Therefore, it could be concluded that prior to apatite coating using alternate soaking process, the titanium substrate should be subjected to a combined pretreat-

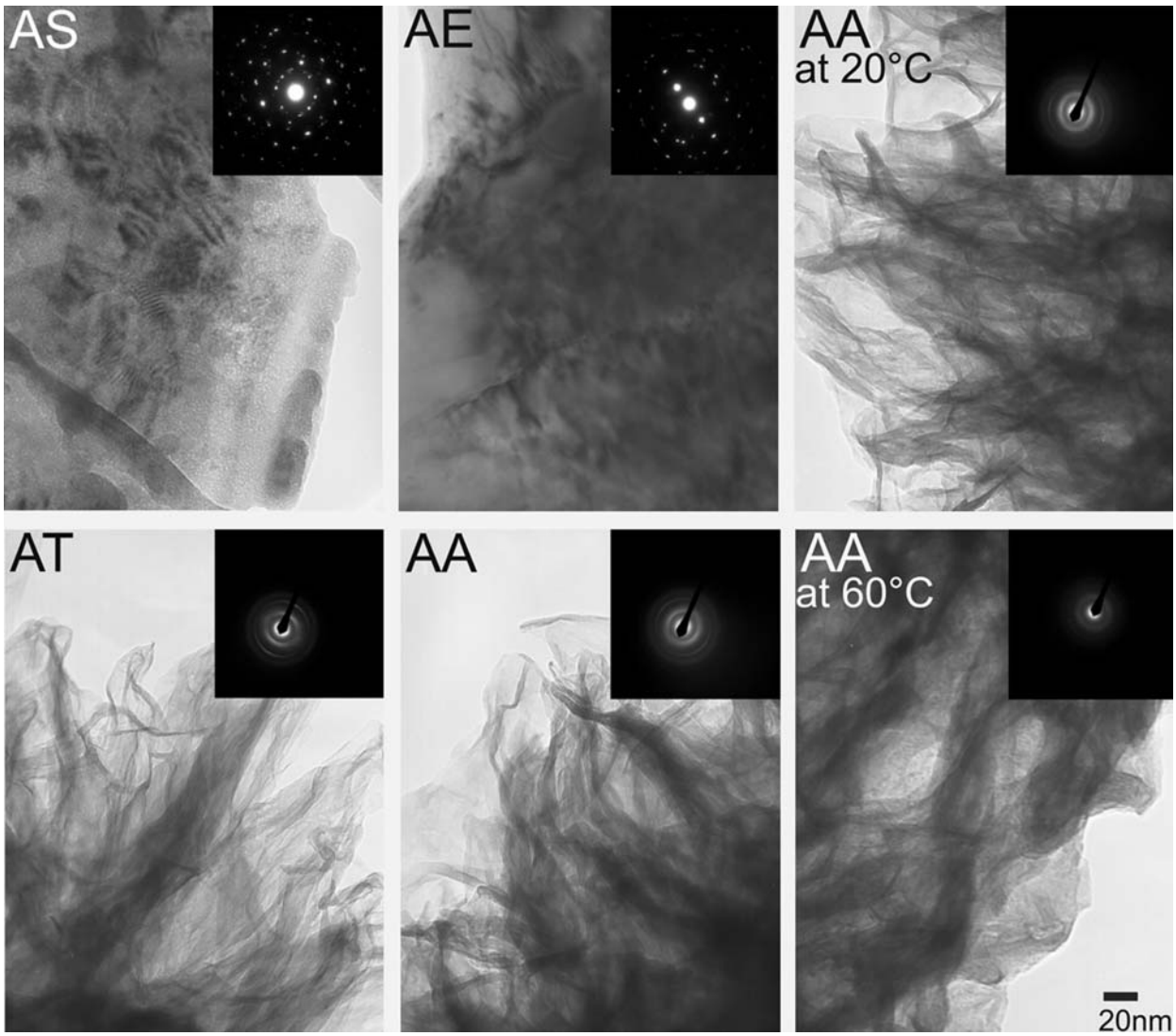

Fig. 11 TEM images and electron diffraction patterns of AS, AE, AT, and AA before and after one reaction cycle at 20 and 60℃ of the 200-grid cpTi mesh.

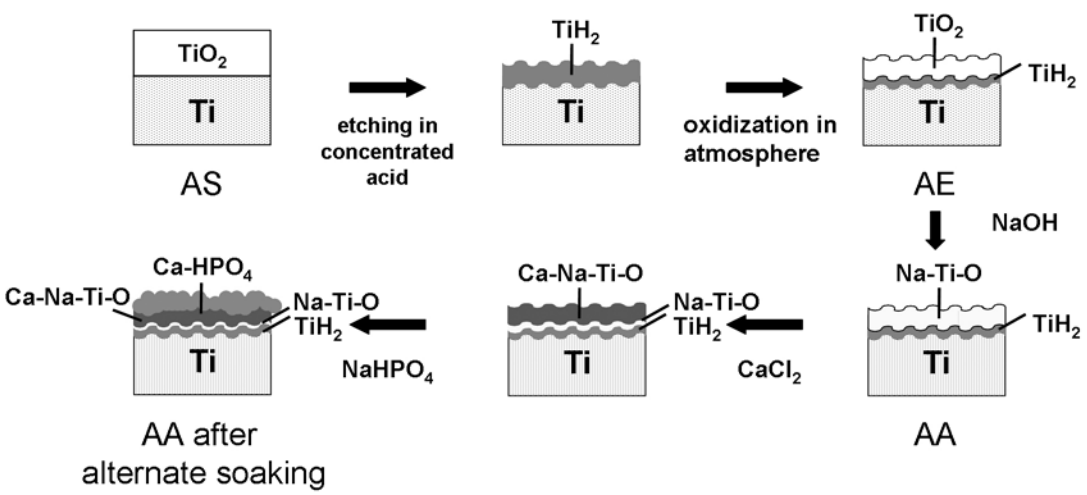

Fig. 12 Schematic drawing of possible structural changes of Ti surface after pretreatment and alternate soaking.

ment of acid etching and alkaline treatment.

The present study demonstrated that the deposited amount of apatite using alternate soaking strongly increased with the number of reaction cycles, but was independent of solution temperature and soaking time. As shown in Fig. 8, the deposited apatite was independent of solution temperature. This was an unexpected result. It is known that the solubility of calcium phosphate salts decreases with solution temperature. The relation between the solubility product, *Ks*, for hydroxyapatite and the solution temperature, *T*, is given as follows[25]:

$$\mathrm{Log}\ Ks = -8219.41/T - 1.6657 - 0.09825T \quad (1)$$

where *T* is in Kelvin and *Ks* in mol/L[9].

According to Equation (1), the solubility product at 4, 37, and 60℃ could be given as 2.177×10^{-59}, 1.844×10^{-59}, and 6.983×10^{-60} mol/L[9], respectively. In other words, the solubility of hydroxyapatite at 4℃ was about three times larger than that at 60℃. However, the deposited amounts were nearly the same among them. This seemed to be related to the crystal phase and crystallinity. The deposits at 4℃ were almost amorphous, judging from the XRD pattern (Fig. 5), whereas the deposited amount at 4℃ was nearly the same as that at 37 and 60℃ (Fig. 8). Therefore, it seemed that the solubility product of hydroxyapatite was not applicable to the deposits at 4℃ in this study. Furthermore, it could be that the apatite coating mainly depended on surface reaction, but not on precipitation in the solution. As shown in Fig. 7, Ca/P ratio increased with the number of reaction cycles and was close to the ratio of stoichiometric hydroxyapatite. Although the solution contained Ca^{2+}, Cl^{-}, Na^{+}, and HPO_4^{2-}, EDX results confirmed that the deposits consisted of Ca, P, and O, and neither Cl nor Na was detected (Fig. 6). This could be due to the solubility of each reaction product. Calcium orthophosphates such as $Ca_3(PO_4)_2$ and $Ca_{10}(PO_4)_6(OH)_2$ are rarely soluble, but other compounds such as $CaCl_2$, NaCl and Na_2HPO_4 are well soluble[26].

The deposited amount of apatite mainly depended on the number of reaction cycles. In the case of apatite formation in the poly(vinylalcohol) (PVA) gel[10], the deposited amount of apatite increased almost linearly with an increase in the number of reaction cycles and the swelling ratio of PVA. It implied that apatite formation by alternate soaking depended on the hydrophilic groups on the substrate surface. In fact, all the previous reports describing the success of apatite formation using the alternate soaking process were related to formation on hydrophilic substrates, such as silk[27], tendon[28], silica gel[29], poly(vinylalcohol)[30], agarose gel[31], and polymer-grafted polyethylene films[10]. Therefore, the first step of apatite formation by alternate soaking is adsorption of calcium ions or phosphate ions on the hydrophilic surface. Furthermore, the amount of adsorbed ions may depend on the amount of hydroxyl groups. In the present study, the hydroxyl groups on the titanium substrate formed by the combined pretreatment of acid etching and alkaline treatment was thinner than the abovementioned substrates. As a result, the deposited amount of apatite was small and independent of soaking time, because the ions were adsorbed only to the surface, but did not penetrate the inside layer. According to the TEM images (Fig. 11), thickness of sodium titanate was less than 1 nm.

According to the results mentioned above, the possible structural changes of titanium surface after pretreatment and alternate soaking, thereby leading to apatite formation, are shown schematically in Fig. 12. Etching of Ti in the concentrated H_2SO_4 involved the following reactions[32]:

$$TiO_2 + 2H_2SO_4 \rightarrow Ti(SO_4)_2 + 2H_2O \quad (2)$$

$$Ti + 2H_2SO_4 \rightarrow Ti(SO_4)_2 + 2H_2\uparrow \quad (3)$$

$$Ti + H_2 \rightarrow TiH_2 \quad (4)$$

On the TiH_2 layer, a new oxide layer was immediately formed upon contact with air moisture[1]. After soaking in NaOH, the passive oxide layer dissolved to form sodium titanate with low crystallinity.

$$TiO_2 + NaOH \rightarrow HTiO_3^{-} + Na^{+} \quad (5)$$

When this pretreated titanium was soaked in the $CaCl_2$ solutions, Na^{+} ions in the sodium titanate layer were exchanged by H_3O^{+} from the surrounding solution resulting in Ti-OH layer. Simultaneously, Ca^{2+} ions were incorporated in the hydrated Ti-OH layer. Then, upon contact with the phosphate ion-containing solution, Ca^{2+} ions were bound to the negatively charged HPO_4^{2-} or PO_4^{3-} to form calcium phosphate compounds, such as hydroxyapatite or TCP. These reactions were repeated in each alternate soaking cycle, indicating sequential apatite formation.

In conclusion, apatite formation using alternate soaking basically depended on ion exchange and adsorption on the pretreated surface. Further, it was also shown that alternate soaking was a feasible bioactive apatite coating method for titanium substrates with complicated shapes.

ACKNOWLEDGEMENTS

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