

Positively charged bioactive Ti metal prepared by simple chemical and heat treatments

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A highly bioactive bone-bonding Ti metal was obtained when Ti metal was simply heat-treated after a common acid treatment. This bone-bonding property was ascribed to the formation of apatite on the Ti metal in a body environment. The formation of apatite on the Ti metal was induced neither by its surface roughness nor by the rutile phase precipitated on its surface, but by its positively charged surface. The surface of the Ti metal was positively charged because acid groups were adsorbed on titanium hydride formed on the Ti metal by the acid treatment, and remained even after the titanium hydride was transformed into titanium oxide by the subsequent heat treatment. These results provide a new principle based on a positively charged surface for obtaining bioactive materials.

Keywords: bioactive Ti metal; apatite formation; surface charge; chemical treatment; dental implant; orthopaedic implant

1. INTRODUCTION

In general, synthetic materials implanted into bone defects become encapsulated by a fibrous tissue to isolate them from the surrounding bone. Only a few types of ceramic, based on calcium phosphate, have been shown to bond to living bone without the intervention of fibrous tissue at the interface (Kokubo 2008). These are called bioactive ceramics and they are already used clinically as important bone substitutes. However, they are brittle and have poor fracture toughness, and, hence, cannot be used under load-bearing conditions.

In the orthopaedic and dental fields, metallic materials, such as Ti metal and its alloys, are widely used as various implants because of their high fracture toughness and good biocompatibility. However, they do not bond to living bone. As-polished or as-abraded Ti metal is encapsulated by fibrous tissue that isolates it from the surrounding bone when it is implanted into a bone defect (Yan *et al.* 1997; Hacking *et al.* 2002). When a rough texture is produced on its surface by grit blasting or acid etching, Ti can form a direct contact with living bone (Hacking *et al.* 2002). Therefore, some orthopaedic and dental implants have been subjected to grit blasting and/or acid etching (Coelho

et al. 2009). However, this direct contact itself does not bond the implant to bone. To enable Ti metal and its alloys to bond to bone, hydroxyapatite has been coated on their surfaces using various methods (Leeuwenburgh *et al.* 2008). However, the hydroxyapatite coating is not stable in the living body for long periods.

Early on, we found that a titania gel prepared using a sol-gel method forms a bone-like apatite layer on its surface in an acellular simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma (Li *et al.* 1994). On the other hand, it has been shown for various bioactive ceramics that a material able to form bone-like apatite on its surface in an SBF generally forms the apatite on its surface also in the living body and bonds to living bone through the apatite layer (Kokubo & Takadama 2006). Based on these findings, it was assumed that even Ti metal could form apatite on its surface in the living body and bond to living bone through this apatite layer if it could be modified with a functional group that was effective for the nucleation of apatite on its surface. As expected, it was shown that Ti metal formed with sodium titanate on its surface by an NaOH solution and a subsequent heat treatment induced the formation of apatite on its surface in a living body, so that it can bond to living bone (Kim *et al.* 1996; Yan *et al.* 1997; Nishiguchi *et al.* 2003). This treatment was applied to a porous Ti metal layer of a total artificial hip joint,

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One contribution to a Theme Supplement 'Scaling the heights—challenges in medical materials: an issue in honour of William Bonfield, Part II. Bone and tissue engineering'.

and the obtained bioactive joint has been used successfully clinically in Japan since 2007 (Kawanabe *et al.* 2009).

However, the apatite-forming ability of the NaOH- and heat-treated Ti metal is liable to decrease when it is stored in a humid environment for long periods, since the sodium ions of the sodium titanate are slowly released via exchange with the H_3O^+ ions in the moisture in the atmosphere. In this study, we show that a highly bioactive Ti metal formed with titanium oxide on its surface, which is stable in a humid environment, is obtained using only a simple heat treatment after a common acid treatment, and that a new principle for obtaining bioactive materials can be proposed based on the mechanism of apatite formation on the bioactive Ti metal.

Several papers have been published on the formation of apatite on the titanium oxide formed on Ti metal after a chemical treatment. Uchida *et al.* (2002) first reported that Ti metal which formed anatase and rutile on its surface by an NaOH solution, water and a heat treatment induced the formation of apatite on its surface in an SBF. Wang *et al.* (2002) reported that Ti metal which formed anatase on its surface by an $\text{H}_2\text{O}_2/\text{HCl}$ solution and a heat treatment induced the formation of apatite on its surface in an SBF. Wu *et al.* (2004) reported that Ti metal which formed anatase and rutile on its surface by HF/HNO_3 and subsequent $\text{H}_2\text{O}_2/\text{TaCl}_5$ solution treatments induced the formation of apatite on its surface in an SBF. Yang *et al.* (2004) reported that Ti metal which formed anatase and rutile on its surface by anodic oxidation using a spark discharge in an H_2SO_4 solution induced the formation of apatite on its surface in an SBF. Lu *et al.* (2007) reported that Ti metal that was treated with strong nitric acid for a long period induced the formation of apatite on its surface in an SBF, although no titanium oxide was detected on its surface. Lu *et al.* (2008) reported that acid-etched Ti metal which formed rutile on its surface by a heat treatment induced the formation of apatite on its surface in an SBF. Pattanayak *et al.* (2009) reported that the apatite-forming ability of Ti metal with rutile and anatase on its surface after soaking in NaOH and HCl solutions, and then a heat treatment, increased with increasing concentration of the HCl solution. However, there is no consensus on the principles governing the apatite-forming ability of titanium oxide on Ti metal.

2. MATERIAL AND METHODS

2.1. Preparation of the Ti metal samples

Commercial pure Ti metal (Kobe Steel, Japan, grade 2, O_2 content = 0.15 wt%) was cut into rectangular samples (dimensions = $10 \times 10 \times 1 \text{ mm}^3$), and abraded with a no. 400 diamond abrasive plate. All the abraded samples were washed with acetone, 2-propanol and ultrapure water for a period of 30 min each in an ultrasonic cleaner, and then dried overnight in an oven at 40°C .

The samples were soaked in 20 ml of a mixture of 66.3 per cent H_2SO_4 (w/w) solution (Kanto Chemical Co., Inc., Japan) and 10.6 per cent HCl (w/w) solution

(Kanto Chemical Co., Inc.) in a weight ratio of 1:1 at 70°C for a period of 1 h in an oil bath shaken at $120 \text{ strokes min}^{-1}$, then gently washed with ultrapure water and dried overnight in an oven at 40°C . This mixed acid solution is the same etchant used for one of the commercial dental implants (Takeuchi *et al.* 2005).

The acid-treated samples were heated to temperatures in the range $400\text{--}800^\circ\text{C}$ at a rate of 5°C min^{-1} in an Fe–Cr furnace in air. The samples were kept at the desired temperature for a period of 1 h, and then allowed to cool to room temperature at the natural rate of the furnace. As a reference, the abraded samples were heat-treated using the same method without being subjected to an acid treatment.

2.2. Examination of the apatite-forming ability of Ti metal in an SBF

The samples subjected to the acid and heat treatments, and those subjected to only the heat treatment, were soaked in 30 ml of an acellular SBF with ion concentrations nearly equal to those of human blood plasma at 36.5°C ($\text{Na}^+ = 142.0$, $\text{K}^+ = 5.0$, $\text{Mg}^{2+} = 1.5$, $\text{Ca}^{2+} = 2.5$, $\text{Cl}^- = 147.8$, $\text{HCO}_3^- = 4.2$, $\text{HPO}_4^{2-} = 1.0$ and $\text{SO}_4^{2-} = 0.5 \text{ mM}$; Kokubo & Takadama 2006). The SBF was prepared by dissolving reagent grade NaCl, NaHCO_3 , KCl, $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, CaCl_2 and Na_2SO_4 (Nacalai Tesque Inc., Japan) into ultrapure water, and then buffering the solution at $\text{pH} = 7.4$ using tris-hydroxymethylaminomethane ($(\text{CH}_2\text{OH})_3\text{CNH}_2$) and 1 M HCl (Nacalai Tesque Inc.). The samples were removed from the SBF after 1 day, gently washed with ultrapure water and dried in an oven at 40°C . The formation of apatite on the sample surface was examined using scanning electron microscopy (SEM) and thin film X-ray diffraction (TF-XRD) employing the methods described in the next section.

To examine the stability of the apatite-forming ability in a humid environment, the acid- and heat-treated samples were kept under an atmosphere of 95 per cent relative humidity at 80°C for a period of one week, and the formation of apatite on the surface in an SBF was examined using SEM and TF-XRD.

2.3. Analysis of the surface of the Ti metal samples

2.3.1. Scanning electron microscopy. The surface and cross-sectional area of the Ti metal samples subjected to both the acid and heat treatments, to the heat treatment alone, and those subsequently soaked in an SBF were coated with a Pt/Pd film and observed under a field-emission scanning electron microscope (FE-SEM; S-4300, Hitachi Co., Japan), using an acceleration voltage of 15 kV.

2.3.2. Thin film X-ray diffraction. The surface of the Ti metal samples subjected to both the acid and heat treatments, to the heat treatment alone, and those subsequently soaked in an SBF were analysed using TF-XRD (model RNT-2500, Rigaku Co., Japan),

employing a $\text{CuK}\alpha$ X-ray source operating at 50 kV and 200 mA. The glancing angle of the incident beam was set to an angle of 1° against the sample surface.

2.3.3. Surface roughness measurements. The surface roughness of the Ti metal samples subjected to the acid and heat treatments was measured using a surface roughness testing system (Surftest model SV-2000, Mitutoyo Co., Japan) using a stylus with a diameter of 2 μm . Based on the data in the JIS standard 1994, the measuring length, evaluation length and cut-off wavelength used were 2.5, 12.5 and 2.5 mm, respectively. Seven measurements were performed for each sample and averaged.

2.3.4. Scratch resistance measurements. The scratch resistance of the surface layer formed on the Ti metal samples by the acid and heat treatments or the heat treatment alone was measured using a thin film scratch tester (model CSR-2000, Rhescra Co., Japan) employing a stylus with a diameter of 5 μm with a spring constant of 200 g mm^{-1} . Based on the data in the JIS R-3255 standard, the amplitude, scratch speed and loading rate used were 100 μm , 10 $\mu\text{m s}^{-1}$ and 100 mN min^{-1} , respectively. Eight to 10 measurements were carried out for each sample, and the average value was used in our analysis.

2.3.5. Zeta potential measurements. Titanium metal plates (size = $13 \times 33 \times 1 \text{ mm}^3$) were prepared using the same method described in §2.1, and these were subjected to the acid and heat treatments, or to the heat treatment alone, at various temperatures. The volume of the acid solution was increased to 30 ml in the acid treatment, because the surface area of the sample was larger than that used for the other surface analysis measurements. Thus, the treated Ti metal samples were grounded to allow for leakage of any stray charge, and they were immediately set in a zeta potential and particle size analyser (model ELS-Z1, Otsuka Electronics Co., Japan) using a glass cell for the plate sample. The zeta potentials of the samples were measured under an applied voltage of 40 V in a 10 mM NaCl solution. The dispersant monitoring particles of polystyrene latex (size = 500 nm) were coated with hydroxyl propyl cellulose. Five samples were measured for each experimental condition and the average reported in our analysis.

2.3.6. X-ray photoelectron spectroscopy. The surfaces of the Ti metal samples soaked in an SBF for various periods after the acid and heat treatments, or the heat treatment alone, were analysed using X-ray photoelectron spectroscopy (XPS, ESCA-3300KM, Shimadzu Co., Japan) as a function of the soaking time in the SBF. In our analysis, $\text{MgK}\alpha$ radiation ($\lambda = 9.8903 \text{ \AA}$) was used as the X-ray source. The XPS take-off angle was set at 45° , which enabled the system to detect photoelectrons to a depth of 5–10 nm from the surface. The binding energies of the measured spectra were calibrated with reference to the

C_{1s} peak of the surfactant CH_2 groups on the substrate at 284.6 eV.

2.3.7. Radio frequency (RF) glow discharge optical emission spectroscopy. The depth profiles of various elements on the surface of the Ti metal samples subjected to the acid and heat treatments were analysed using RF glow discharge optical emission spectroscopy (GD-OES, GD-Profler 2, Horiba Co., Japan) under Ar sputtering at an Ar pressure of 600 Pa. A RF electric field with a power of 35 W was applied at a regular interval of 20 ms.

2.4. Examination of the bone-bonding ability of Ti metal

The Animal Research Committee of the Graduate School of Medicine, Kyoto University, Japan, approved our animal studies. Rectangular samples (size = $15 \times 10 \times 2 \text{ mm}^3$) of Ti metal subjected to the acid and heat treatments, or the heat treatment alone, were sterilized with ethylene oxide gas and implanted into metaphyses of the tibiae of mature male Japanese white rabbits weighing 2.8–3.2 kg. The surgical methods used have been described previously (Nakamura *et al.* 1985). After four weeks' implantation, the rabbits were sacrificed using an overdose of intravenous pentobarbital sodium. The segments of the proximal tibiae metaphyses containing the implanted samples were retrieved. The bone tissue surrounding the implants was removed on both sides and at the ends using a dental burr. Traction was applied vertically to the sample surface at a crosshead speed of 35 mm min^{-1} using an Instron-type autograph (model 1011, Aikon Engineering Co., Japan). The detaching failure load was recorded when the sample was detached from the bone. Five measurements were performed for each experimental condition and the average value recorded.

Other segments of the tibiae containing the implanted samples were fixed in 10 per cent phosphate-buffered formalin, dehydrated using ethanol, and embedded in polyester resin. Sections with a thickness of 500 μm were cut, bound to a transparent acrylic plate, and ground to a thickness of 40–50 μm . These samples were stained using Stevenel's blue and Van Gieson's picro-fuchsin. A histological evaluation was performed on each stained section using transmitted light microscopy (model Eclipse 80i, Nikon Co., Japan).

3. RESULTS

3.1. Change in the surface structure of the Ti metal after the acid and heat treatments

Figure 1 shows FE-SEM photographs of the surfaces of Ti metal samples as-abraded, and after being heat-treated at 600°C and 800°C . The abraded sample had a smooth surface, but its surface roughness increased a little with increasing temperature of the heat treatment.

Figure 2 shows FE-SEM photographs of the surfaces of Ti metal samples that had been subjected to the acid

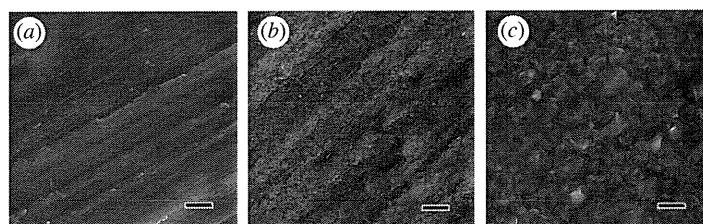


Figure 1. FE-SEM photographs of surfaces of Ti metals as-abraded, and subsequently heat-treated at different temperatures. (a) Before heat treatment, (b) heat-treated at 600°C and (c) heat-treated at 800°C. Scale bars, 1 μm .

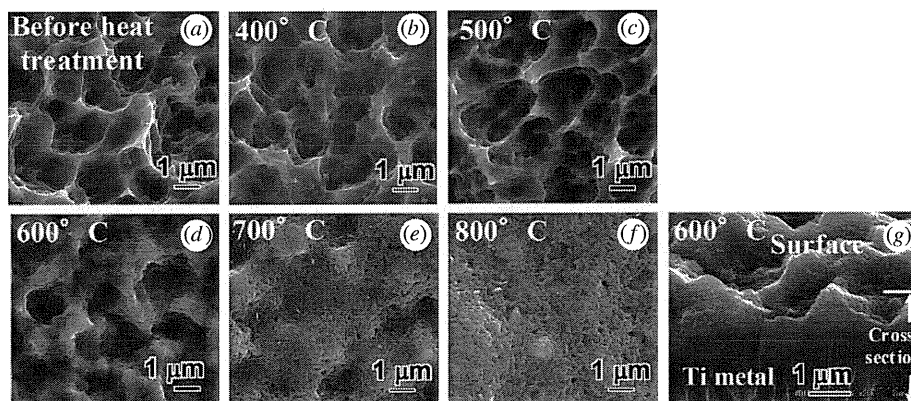


Figure 2. FE-SEM photographs of surfaces and a cross section of Ti metals as acid-treated, and subsequently heat-treated, at various temperatures. (a) Before heat treatment and heat-treated at (b) 400°C, (c) 500°C, (d) 600°C, (e) 700°C, (f) 800°C and (g) a cross section of Ti metal at 600°C.

treatment and subsequently heat-treated at various temperatures up to 800°C. A cross-sectional view of a sample heat-treated at 600°C is also shown in figure 2. A surface roughness was produced on the Ti metal sample by the acid treatment, and this remained unchanged up to 600°C, but the sample surface roughness decreased above 700°C.

According to our surface roughness measurements, the values of R_a and R_z were $R_a = 0.99 \pm 0.07$ and $R_z = 8.87 \pm 0.94$ μm for the samples that had been acid-treated, and $R_a = 0.99 \pm 0.17$ and $R_z = 7.33 \pm 0.83$ μm for the samples that had been heat-treated at 600°C.

The scratch resistance of the Ti metal samples as-abraded and acid-treated were very low, about 1–2 mN. Both of these samples showed a marked increase up to 40–55 mN after the subsequent heat treatments at 600–800°C.

Figure 3*a, b* shows thin film X-ray diffraction patterns of the surfaces of the Ti metal samples subjected to heat treatment at various temperatures, without and after the acid treatment, respectively. The as-abraded sample consisted of α titanium, whereas the acid-treated sample formed a titanium hydride layer (TiH_x , where $0 < x < 2$) on its surface. Despite these differences, both of these samples began to precipitate rutile around 500°C on their surfaces, and the rutile content increased with increasing heat-treatment temperature up to 800°C. It is apparent from these results that the increase in surface roughness of the abraded sample, the decrease in surface roughness of the acid-treated sample, and the increase in the scratch resistance of

the abraded and acid-treated samples with increasing temperature of the heat treatment are all attributed to the precipitation of the rutile on the surface of the Ti metals.

3.2. Change in the apatite-forming ability of Ti metal in an SBF after acid and heat treatments

Figure 4 shows FE-SEM photographs of the surface of Ti metal samples soaked in an SBF for a period of 1 day, after being abraded and subsequently heat-treated at 600 and 800°C. No deposit was observed on these surfaces.

Figure 5 shows FE-SEM photographs of the surfaces of Ti metal samples soaked in an SBF for a period of 1 day, after being treated with acid and subsequently heat-treated at various temperatures up to 800°C. The cross section of a sample soaked in an SBF for a period of 1 day after the acid and heat treatments at 600°C is also shown in figure 5.

Figure 6 shows TF-XRD patterns of the surfaces of Ti metal samples soaked in an SBF for a period of 1 day, after being treated with acid and subsequently heat-treated at various temperatures. The round particles observed on the surfaces of the Ti metal samples in figure 5 are identified as being apatite from TF-XRD patterns of figure 6, although its detailed structure is not clear from these TF-XRD patterns (Lu & Leng 2004). From figure 5, it can be seen that apatite was deposited actively only on the surface of Ti metal

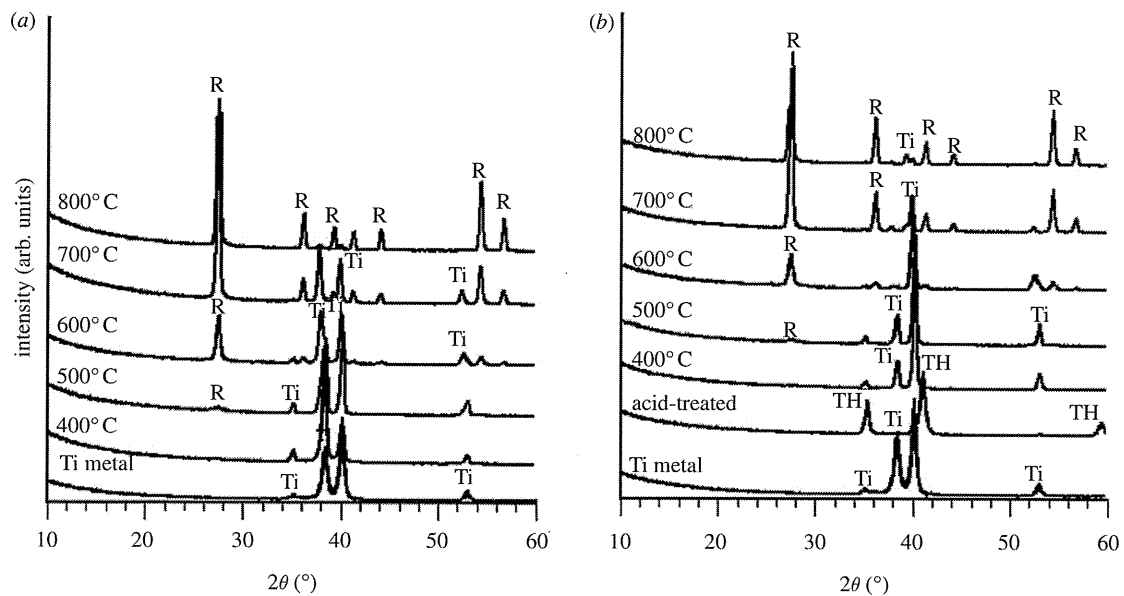


Figure 3. TF-XRD patterns of surfaces of Ti metals heat-treated at various temperatures (a) without and (b) after acid treatment. R, rutile; Ti, α titanium; TH, TiH_x.

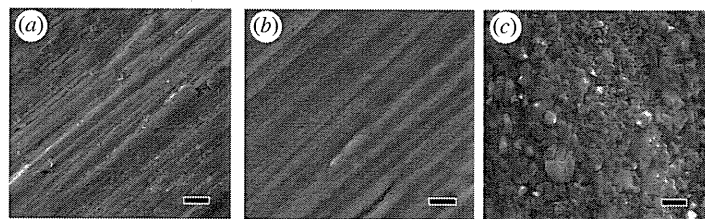


Figure 4. FE-SEM photographs of surfaces of the Ti metals soaked in SBF for 1 day, after being abraded, and subsequently heat-treated at different temperatures. (a) Before heat treatment, (b) heat-treated at 600°C and (c) heat-treated at 800°C. Scale bars, 2 μ m.

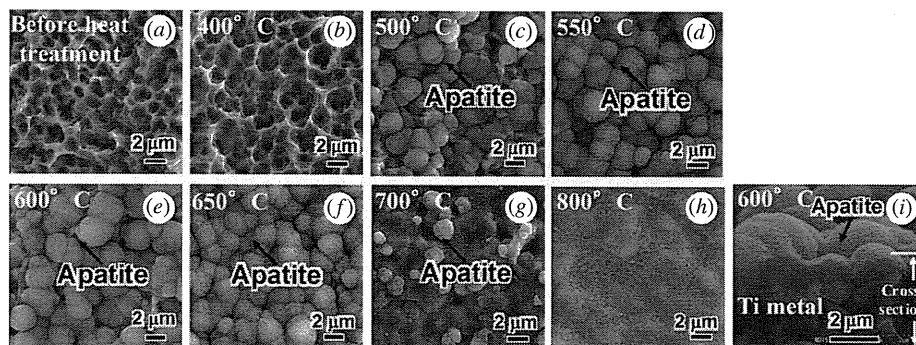


Figure 5. FE-SEM photographs of surfaces and a cross section of Ti metals soaked in SBF for 1 day, after being heat-treated at various temperatures following the acid treatment. (a) Before heat treatment and heat-treated at (b) 400°C, (c) 500°C, (d) 550°C, (e) 600°C, (f) 650°C, (g) 700°C, (h) 800°C and (i) a cross section of Ti metal at 600°C.

samples that had been heat-treated at temperatures from 500 to 650°C after an acid treatment.

Figure 7 shows an FE-SEM photograph of the surface of a Ti metal sample soaked in an SBF for a period of 1 day that had been kept under an atmosphere of 95 per cent relative humidity at 80°C for a period of one week following an acid and heat treatment at 600°C. The apatite-forming ability of the acid- and heat-treated Ti metal sample was only a little decreased

even after it was kept in a humid environment for a period of one week.

3.3. Change in the zeta potential of Ti metal after acid and heat treatments

Figure 8 shows the zeta potentials of the surfaces of Ti metal samples heat-treated at various temperatures without and after an acid treatment. The zeta

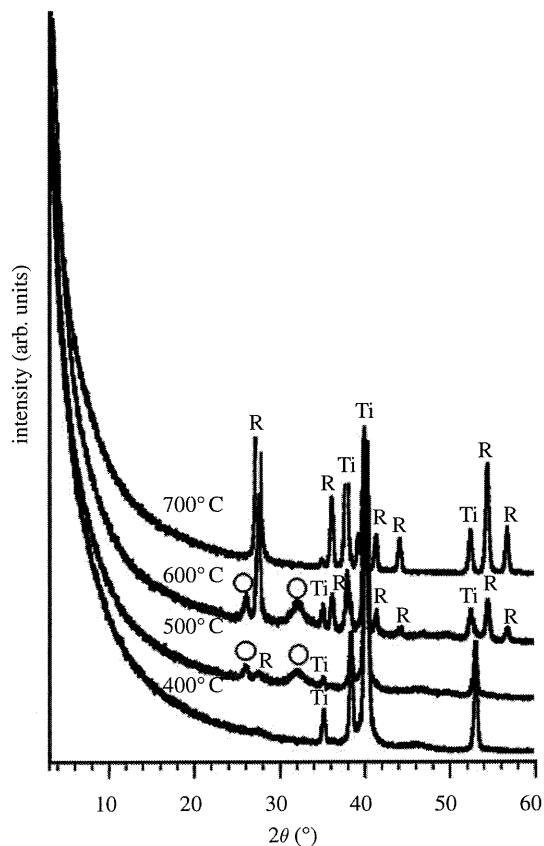


Figure 6. TF-XRD patterns of the surfaces of Ti metals soaked in SBF for 1 day after being heat-treated at various temperatures following acid treatment. Ti, α titanium; R, rutile; open circle, apatite.

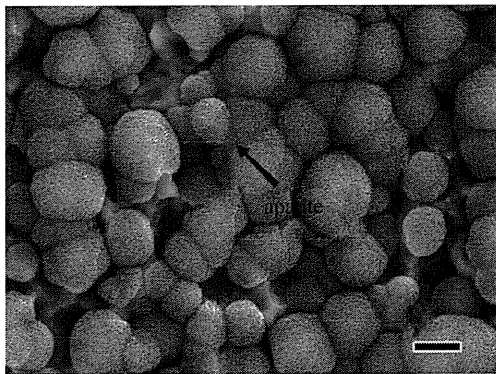


Figure 7. FE-SEM photograph of the surface of Ti metal soaked in SBF for 1 day, after being kept in a humid environment for one week following the acid and heat treatments at 600°C (scale bar, 2 μ m).

potentials of the samples that had been heat-treated at temperatures lower than 500°C were not able to be measured, since there was no or only a thin insulating titanium oxide layer formed on their surface (see figure 3). The Ti metal samples heat-treated without being subjected to the acid treatment showed low zeta potentials, around zero, irrespective of the temperature of the heat treatment, whereas those heat-treated after

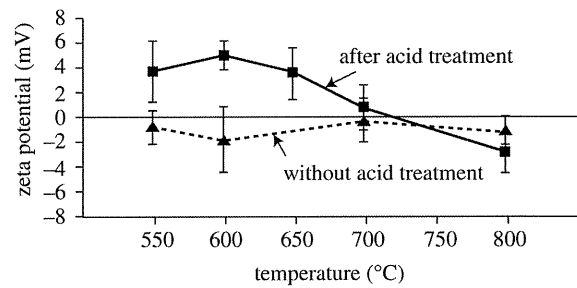


Figure 8. Zeta potentials of surfaces of Ti metals heat-treated at various temperatures without and after acid treatment.

the acid treatment showed positive zeta potentials when heat-treated at temperatures in the range 550–650°C.

3.4. Change in the XPS spectra of Ti metal after acid and heat treatments

Figure 9 shows the XPS spectra of the surfaces of the Ti metal samples as-abraded and subsequently heat-treated at 600°C as a function of the soaking time in the SBF. The as-abraded sample adsorbed only a small amount of the calcium and phosphate ions, almost simultaneously, on its surface, even after 12 h in the SBF, and this property was unchanged after heat treatment at 600°C.

Figure 10 shows the XPS spectra of the surfaces of the Ti metal samples that had been treated with acid and subsequently heat-treated at 600°C as a function of the soaking time in the SBF. The acid-treated sample also adsorbed only a small amount of the calcium and phosphate ions, almost simultaneously, in the SBF, whereas the sample heat-treated at 600°C after the acid treatment initially adsorbed a large amount of phosphate ions preferentially on its surface, and then later adsorbed a large amount of calcium ions.

3.5. Change in the GD-OES spectra of Ti metal after acid and heat treatments

Figure 11 shows the depth profile of the GD-OES spectra of Ti metal samples that were treated with acid, subsequently heat-treated at 600°C, and then kept under an atmosphere of 95 per cent relative humidity at 80°C for a period of one week. Large amounts of H and O, besides Ti atoms, were detected in the surface layer of the samples that were treated with acid and subsequently heat-treated, respectively, because of the presence of titanium hydride and titanium oxide on their surfaces. Besides the presence of H, O and Ti, a large amount of Cl and S atoms was detected in the surface layer of the samples that had been treated with acid, and a considerable amount of these atoms was detected even after the subsequent heat treatment at 600°C and being kept in a humid environment for a period of one week.

3.6. Bonding of Ti metal to bone

The as-abraded titanium metal samples and those subsequently heat-treated at 600°C showed a detaching failure load of 1.86 ± 1.72 and 6.12 ± 3.43 N,

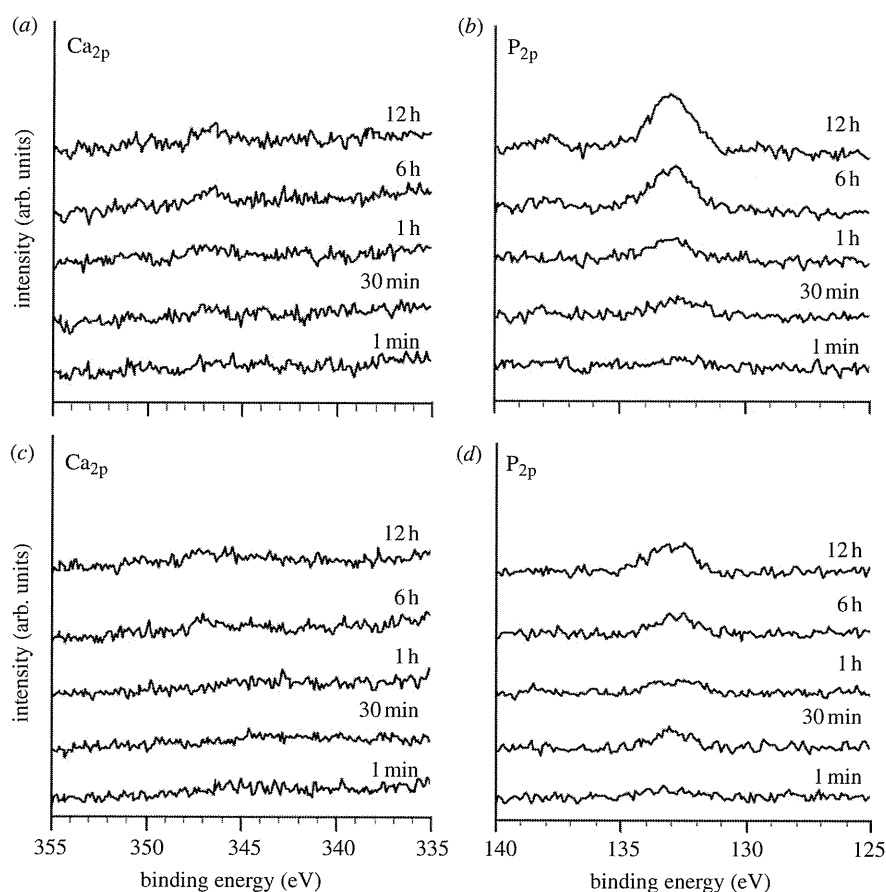


Figure 9. (a,b) XPS spectra of the surfaces of Ti metals abraded and (c,d) subsequently heat-treated at 600°C, as a function of soaking time in SBF.

respectively, when they were implanted into the tibia of a rabbit and subjected to the detaching test at four weeks after implantation. The acid-treated samples and those subsequently heat-treated at 600°C showed a failure load of 4.91 ± 1.94 and 13.3 ± 4.67 N, respectively. The failure load of the abraded sample was only slightly increased by the heat treatment or acid treatment. However, it was increased markedly by a heat treatment after the acid treatment.

Figure 12 shows the light micrographs of an interface with the living bone of a sample that was heat-treated at 600°C after the acid treatment. Bone had grown along the surface of the Ti metal sample and it was in direct contact with the sample without the intervention of any fibrous tissue.

4. DISCUSSION

It is apparent from the experimental results described above that a high apatite-forming ability in an SBF is conferred on Ti metal by neither a heat treatment alone nor an acid treatment alone, but is conferred by a heat treatment after an acid treatment. Titanium metal subjected to a heat treatment after an acid treatment to confer a high apatite-forming ability was confirmed to be directly in contact with newly grown bone and formed tight bonding to the bone in the

animal experiments. This means that bioactive Ti metal can be obtained by a simple heat treatment after a common acid treatment. The high apatite-forming ability of our prepared Ti metal was confirmed to be maintained, even when kept in a humid environment for a long period. It was also shown that our prepared Ti metal showed a considerably high scratch resistance. Because of these properties, our prepared bioactive Ti metal is believed to be useful for various types of implant in the dental and orthopaedic fields.

It should be noted here that porous bioactive Ti metal with titanium oxide on its surface after NaOH, HCl and heat treatment exhibits high osteoinductivity, which is poor for porous Ti metal with sodium titanate on its surface after NaOH and heat treatment, in addition to the observed osteoconductivity (Takemoto *et al.* 2006). That is, these samples show active bone formation not only in bone defects but also in muscles. The higher osteoinductivity of the former samples compared with the latter is considered to be attributed to the lack of ions, which, in the latter, are released to form unfavourable conditions for the cells by increasing the pH. In view of this fact, the bioactive Ti metal formed with the titanium oxide on its surface using the present method can also be expected to exhibit a high osteoinductivity. This is now being investigated.

Although detailed results of the animal experiments described in §2.4 will be published elsewhere, the tight

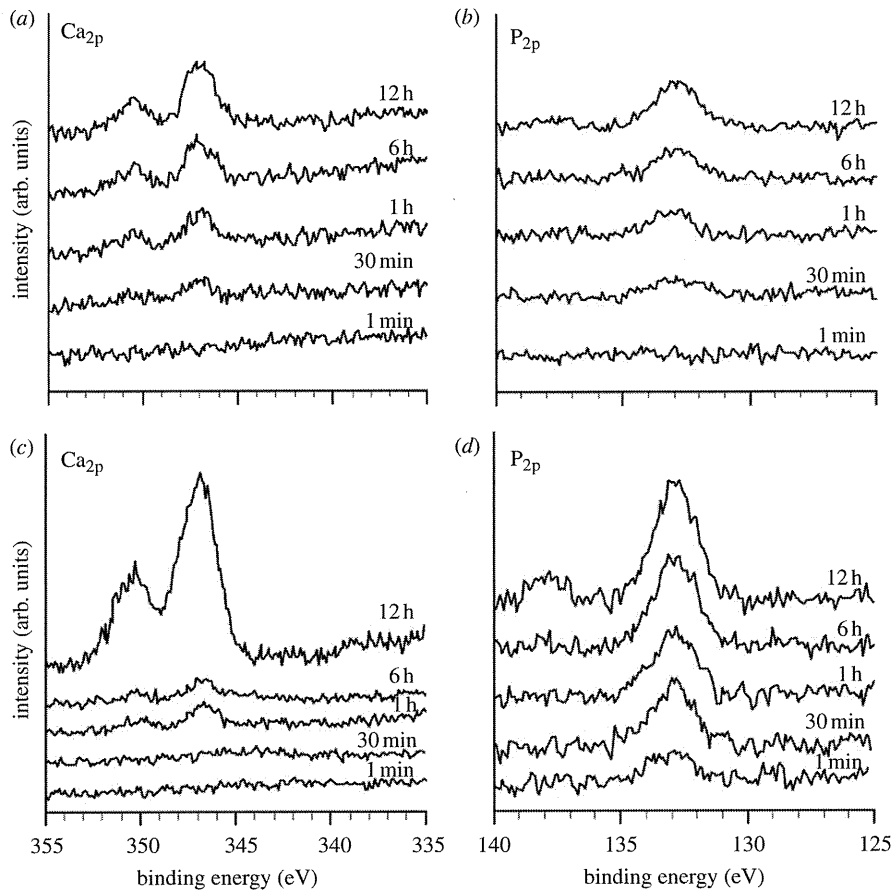


Figure 10. (a,b) XPS spectra of surfaces of Ti metals acid-treated and (c,d) subsequently heat-treated at 600°C, as a function of soaking time in SBF.

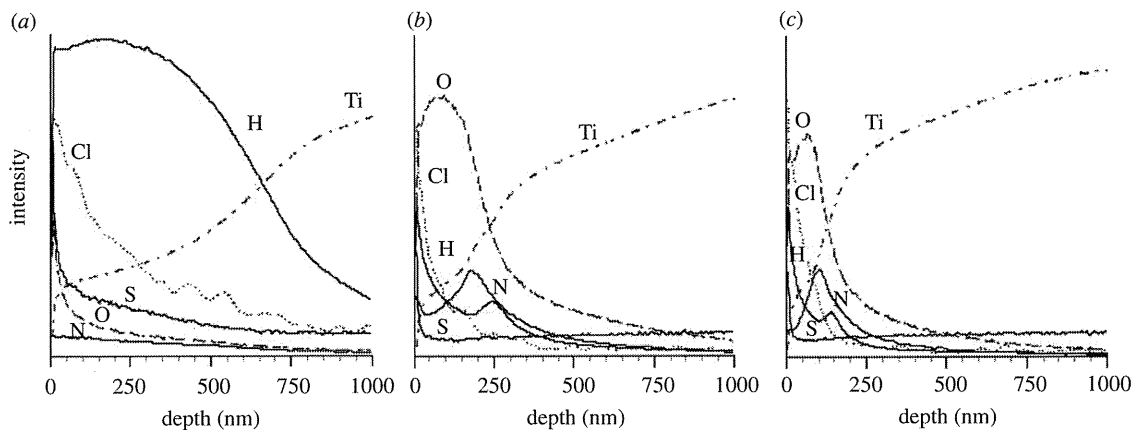


Figure 11. (a) Depth profiles of GD-OES spectra of Ti metals acid-treated, (b) subsequently heat-treated at 600°C and (c) kept in a humid environment.

bonding of the Ti metal heat-treated after the acid treatment can be attributed to the formation of apatite on its surface in the living body. We are concerned as to the reason why Ti metal forms apatite on its surface in a body environment when it is heat-treated at temperatures in the range 500–650°C after an acid treatment. A rough surface was produced on the Ti metal by the acid treatment, but the surface roughness was unchanged in

the temperature range 500–650°C from before heat treatment (see figure 2). Therefore, the formation of apatite on our Ti metal samples cannot be attributed to their surface roughness. The acid-treated Ti metal samples precipitated rutile on their surface when heat-treated at 500–650°C, but the Ti metal samples without an acid treatment also precipitated the same phase in the same temperature range (see figure 3).

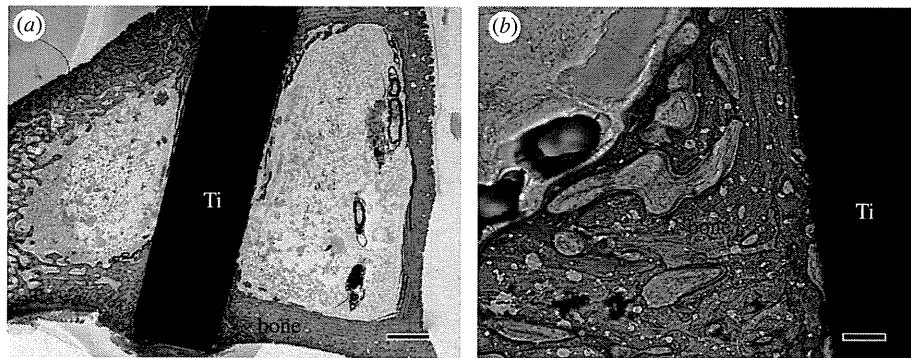


Figure 12. Light micrographs of strained sections of acid- and heat-treated Ti metal implanted into a rabbit tibia for four weeks. Scale bars, (a) 1 mm, (b) 100 μm .

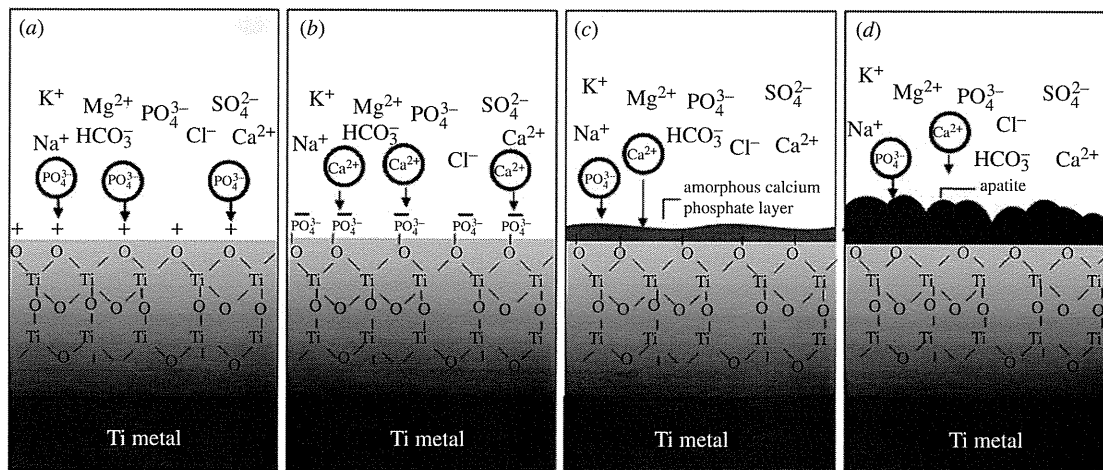


Figure 13. Process of formation of apatite on positively charged Ti metal in SBF. Apatite is formed by the process shown in (a–d).

Therefore, the formation of apatite on our Ti metal samples cannot be attributed to a specific crystalline phase.

The most probable reason for the formation of apatite on our Ti metal samples is their positive surface charge. Figure 8 shows that only the Ti metal samples that were heat-treated at temperatures of 550–650°C after an acid treatment showed a positive zeta potential. Although the zeta potential of Ti metal heat-treated at 500°C after an acid treatment was not measured, it is assumed to have a positive value, since the Ti metal sample heat-treated at 500°C had a titanium oxide layer on its surface, similar to Ti metal samples heat-treated at 550–650°C, even though this layer was very thin (see figure 3). The zeta potentials of the Ti metal samples acid-treated and subsequently heat-treated at temperatures below 400°C also could not be measured, because the insulating titanium oxide layer was not formed on their surfaces (see figure 3). This means that their surfaces are electrically conductive, and hence their zeta potentials are zero.

Our question now is to find the reason why the positively charged titanium oxide on Ti metal forms apatite on its surface in the body environment. The XPS spectra of Ti metal shown in figure 10 show that Ti metal

heat-treated at 600°C after an acid treatment preferentially adsorbs a large amount of phosphate ions first, and then later it also adsorbs calcium ions. This result indicates that positively charged titanium oxide induces the formation of apatite on its surface by the process shown in figure 13. The positively charged titanium oxide first selectively adsorbs the negatively charged phosphate ions on its surface. As the phosphate ions begin to accumulate, the surface becomes negatively charged, and, hence, combines with the positively charged calcium ions to form calcium phosphate. The calcium phosphate formed eventually transforms into stable crystalline apatite.

This process of the formation of apatite on a positively charged titanium oxide layer contrasts with that where sodium titanate is formed on Ti metal after NaOH and heat treatments (Kim *et al.* 2003). In this case, the sodium titanate releases Na^+ ions via exchange with the H_3O^+ ions in the body fluid to form Ti–OH groups on its surface. The Ti–OH groups formed are negatively charged (Kokubo *et al.* 1982), since the pH of the surrounding body fluid is increased by the Na^+ ions released, and, hence, combines with the positively charged Ca^{2+} ions in the body fluid to form calcium titanate. As the Ca^{2+} ions

accumulate, the surface becomes positively charged and combines with the negatively charged phosphate ions to form calcium phosphate. Thus, the calcium phosphate formed eventually transforms into stable crystalline apatite. Ever since discovering this apatite-forming process, it was believed that a negatively charged surface is essential for obtaining bioactive materials. However, the present results show that a positively charged surface can also provide bioactive materials by inducing the formation of apatite. This finding provides a new principle for the development of bioactive materials. Generally, Ti metal exhibits surface charge around zero in a body environment independent of heat treatment, as shown for a Ti metal sample without acid treatment in figure 8, and, hence, does not induce apatite formation (see figure 4). However, the present results indicate that Ti metal is negatively charged on its surface in a body environment if it has been subjected to alkali and heat treatments, and it is positively charged on its surface if it has been subjected to acid and heat treatments, and, hence, it can induce apatite formation on its surface in a body environment to give bone-bonding ability.

The reason why Ti metal heat-treated at 500–650°C after an acid treatment is positively charged is interpreted in terms of figure 11. Acid groups, such as chloride and sulphate ions, were adsorbed onto the surface of, and incorporated into, the titanium hydride layer on the surface of the Ti metal formed during the acid treatment, and a considerable amount of these ions remained in the surface layer, even after the titanium hydride was transformed into titanium oxide by the heat treatment at 600°C. These acid groups could be released from the surface to form an acidic environment in the vicinity of the surface when the Ti metal is soaked in an SBF. As a result, the surface would be positively charged, since titanium oxide is positively charged in an acidic environment (Kokubo *et al.* 1982; Gold *et al.* 1989; Textor *et al.* 2001). The Ti metal samples heat-treated at temperatures lower than 400°C after an acid treatment had no insulating oxide layer on their surface, and, hence, their surface was not charged and so did not induce formation of apatite in an SBF. The Ti metal samples heat-treated at temperatures higher than 700°C after the acid treatment decomposed incorporated ions on heat treatment, and, hence, their surface was not positively charged, and so did not induce the formation of apatite. Figure 11 shows that the incorporated ions almost remain on the surface of the Ti metal heat-treated at 600°C after the acid treatment, even after the Ti metal was kept in a humid environment for a period of one week. As a result, the apatite-forming ability of this Ti metal was only a little decreased, even after it was kept in a humid environment.

These results indicate that the requirement for obtaining a positively charged Ti metal that is effective for inducing the formation of apatite on its surface in a body environment is the formation of an electrically insulating oxide layer on its surface, and the adsorption of acid groups on its surface. These requirements are met only by the common acid treatment and subsequent heat treatment at moderately high temperatures. This indicates that the apatite formation of

the Ti metal heat-treated after acid treatment does not depend upon the kind of acid solutions, but upon the pH of the solutions. This has already been confirmed experimentally and will be published elsewhere.

In view of this fact, most of the chemical and heat treatments for obtaining bioactive Ti metal by forming a titanium oxide on its surface, which were cited in §1, can be interpreted in terms of the method that forms a layer that can meet these requirements on the surface of the Ti metal.

Zhao *et al.* (2005, 2008) reported that a plasma-sprayed TiO₂ coated layer with a rutile phase showed a high apatite-forming ability in an SBF when treated with an acid solution, such as H₂SO₄ and HNO₃. Kokubo *et al.* (2008) reported that a titania gel layer coated on polyethylene terephthalate using a sol-gel method formed apatite on its surface in an SBF when it was treated with an HCl solution at 80°C for a period of 8 days. These results can also be interpreted in terms of the formation of a titanium oxide layer containing adsorbed acid groups on its surface to give a positive surface charge in an SBF.

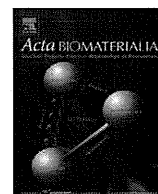
5. SUMMARY

- An ability to form apatite in an SBF was conferred on Ti metal neither by an H₂SO₄/HCl mixed acid treatment alone nor by a heat treatment alone, but by a heat treatment at 500–650°C after the acid treatment. The apatite-forming ability was hardly decreased, even after the Ti metal was maintained in a humid environment for a long period. The acid- and heat-treated Ti metal showed a high scratch resistance. These are important properties for clinical applications of Ti metal.
- Ti metal that was heat-treated at 600°C after the acid treatment was confirmed to be in direct contact with living bone and tightly bonded to it. This tight bonding was attributed to the formation of apatite on the surface of the Ti metal in a body environment.
- The formation of apatite on Ti metal that had been heat-treated at 500–650°C after an acid treatment was attributed neither to the roughness of the surface nor to the rutile phase that had precipitated on the surface, but to the positive charge on the surface.
- The positive charge on the Ti metal was shown to be formed by the adsorption of acid groups on the surfaces by the acid treatment, and by the formation of a non-conducting titanium oxide layer on the surface by the subsequent heat treatment.
- A new principle for obtaining bioactive materials based on a positively charged surface has been proposed.

This work was supported in part by a Grant-in-Aid from the Ministry of Health, Labour, and Welfare of Japan (H21-Trans-Ippan-003) and by a Grant-in-Aid for Scientific Research (A) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (19200039).

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Preparation of bioactive Ti metal surface enriched with calcium ions by chemical treatment

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ARTICLE INFO

Article history:

Received 22 October 2009

Received in revised form 6 January 2010

Accepted 7 January 2010

Available online 13 January 2010

Keywords:

Titanium

Calcium

Apatite

Scratch

Moisture

ABSTRACT

A calcium solution treatment was applied to a NaOH-treated titanium metal to give it bioactivity, scratch resistance and moisture resistance. The titanium metal was soaked in a 5 M NaOH solution and then a 100 mM CaCl₂ solution to incorporate Ca²⁺ ions into the titanium metal surface by ion exchange. This treated titanium metal was subsequently heated at 600 °C and soaked in hot water at 80 °C. The NaOH treatment incorporated ~5 at.% Na⁺ ions into the Ti metal surface. These Na⁺ ions were completely replaced by Ca²⁺ ions by the CaCl₂ treatment. The number of Ca²⁺ ions remained even after subsequent heat and water treatments. Although the NaOH–CaCl₂-treated titanium metal showed slightly higher apatite-forming ability in a simulated body fluid than the NaOH-treated titanium metal, it lost its apatite-forming ability during the heat treatment. However, subsequent water or autoclave treatment restored the apatite-forming ability of the NaOH–CaCl₂-heat-treated titanium metal. Although the apatite-forming ability of the NaOH-heat-treated titanium metal decreased dramatically when it was kept at high humidity, that of NaOH–CaCl₂-heat-water-treated titanium metal was maintained even in the humid environment. The heat treatment increased the critical scratch resistance of the surface layer of the NaOH–CaCl₂-treated titanium metal remarkably, and it did not deteriorate on subsequent water treatment.

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1. Introduction

Titanium (Ti) metal and its alloys have been widely used in medical devices such as artificial joints and dental implants because of their high fracture toughness and good biocompatibility, however, they do not bond to living bone. It is desirable to provide a bone-bonding ability to Ti metal and its alloys. To achieve this, a technique was developed to coat Ti metal and its alloys with calcium phosphate using a plasma spray. However, this method cannot form a uniform bioactive layer on complex shapes, because only the surfaces exposed to the plasma are coated and calcium phosphate is liable to decompose in the living body.

Solutions and thermal treatments can form a uniform bioactive surface layer, even on complex shapes. It has been shown that Ti metal forms a sodium titanate layer on its surface when soaked in NaOH solution at 60 °C for 24 h and then heat treated at 600 °C for 1 h. Ti metal with a surface sodium titanate layer spontaneously forms a bone-like apatite on its surface in the body environment and directly bonds to living bone through this apatite layer [1–5]. These treatments were applied to the porous Ti metal layer of an artificial hip joint commercialized in Japan in 2007 [6]. How-

ever, the apatite-forming ability of NaOH- and heat-treated Ti metal is liable to decrease when the treated-Ti metal is stored in a humid environment for a long period of time, because of the release of Na⁺ ions from the sodium titanate.

Ti metal containing Ca²⁺ ions on its surface, instead of Na⁺ ions, is expected to exhibit a higher apatite-forming ability, since the released Ca²⁺ ions more effectively increase the ionic activity product of the apatite in the surrounding body fluid than released Na⁺ ions [7]. However, calcium ions cannot be incorporated into the surface of Ti metal in the same manner as NaOH ions because of the low solubility of Ca(OH)₂ in water. Many studies on the incorporation of Ca²⁺ ions into Ti metal surfaces have been performed. Nayab et al. [8] reported that Ti metal with incorporated Ca²⁺ ions, incorporated using ion implantation, showed better cell adhesion properties than non-implanted Ti metal. However, implanted Ca²⁺ ions can reach only a shallow area, and the richest Ca²⁺ area exists a small distance below the top surface [9]. Sul [10] showed that about 11 at.% Ca ions were incorporated into a TiO₂ layer ~1300 nm thick on the surface of Ti metal by micro-arc oxidation in a calcium-containing mixed electrolyte system. The resultant dental implant showed a higher removal torque than untreated implant. However, this higher torque cannot be simply attributed to formation of a calcium-containing TiO₂ layer on the Ti metal, as a porous structure with a number of craters is formed on the

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surface of the Ti metal on micro-arc oxidation. Fröjd et al. [11] later reported that a calcium-incorporated titanium oxide layer formed on Ti metal by micro-arc oxidation gives greater bone contact than a calcium-free oxide layer. Song et al. [12] did not observe apatite formation in simulated body fluid (SBF) on a Ca-containing titanium oxide formed by micro-arc oxidation, but did so on a surface layer formed with amorphous $\text{Ca}(\text{OH})_2$ by subsequent hydrothermal treatment with water at 250 °C for 2 h. Incorporation of calcium ions into Ti metal by hydrothermal treatment was also attempted by Nakagawa et al. [13]. They showed that a small number of calcium ions are incorporated into the surface of Ti metal by hydrothermal treatment with CaCl_2 solution at 200 °C for 24 h, and that thus treated Ti metal forms apatite on its surface in SBF. Park et al. [14] incorporated larger numbers of calcium ions into the surface of a Ti–6Al–4V alloy, by forming CaTiO_3 through hydrothermal treatment with NaOH and CaO solution at 180 °C for 24 h. The thus treated alloy also formed apatite on its surface in Hank's solution and showed an increased bone–implant contact. Ueda et al. [15] observed apatite formation in SBF on Ti metal which had first been treated with $\text{H}_2\text{O}_2/\text{HNO}_3$ solution at 80 °C for 60 min and then hydrothermally treated with $\text{Ca}(\text{OH})_2$ solution at 180 °C for 12 h. Chen et al. [16] showed that calcium ions were incorporated into surface of porous Ti metal by hydrothermal treatment with 0.2 M $\text{Ca}(\text{OH})_2$ solution at 250 °C for 8 h.

Both the micro-arc oxidation and hydrothermal treatments described above, however, need an apparatus specially designed to apply an electric field or high pressure to medical devices.

Rakngarm et al. [17] tried to incorporate calcium ions into the surface of Ti metal and its alloys by NaOH and $\text{Ca}(\text{OH})_2$ solution treatments. Thus treated Ti metal and its alloy formed apatite on their surfaces in SBF. However, these metals were not heat treated after the NaOH and $\text{Ca}(\text{OH})_2$ treatments. The resultant products showed neither high scratch resistance nor high apatite-forming ability.

This study attempted the preparation of Ti metal with a surface enriched with calcium ions by simple chemical and thermal treatments and examined the apatite-forming ability and scratch resistance of the resulting products, as well as stability of apatite-forming ability in humid environments.

2. Materials and methods

2.1. Sample preparation

Commercial pure Ti metal (Ti > 99.5%, Nilaco Co., Japan) $10 \times 10 \times 1 \text{ mm}^3$ was polished with a No. 400 diamond disk and then washed in acetone, 2-propanol and ultrapure water with an ultrasonic cleaner for 30 min. The Ti metal was soaked in 5 ml of a 5 M NaOH solution at 60 °C for 24 h and then gently washed with ultrapure water. The NaOH-treated Ti metal was subsequently soaked in 10 ml of 100 mM CaCl_2 solution at 40 °C for 24 h and then gently washed with ultrapure water. The NaOH- and NaOH– CaCl_2 -treated Ti metals were heated to 600 °C at a rate of 5 °C min^{-1} and at 600 °C for 1 h in an air atmosphere. The NaOH– CaCl_2 -heat-treated Ti metal was subsequently subjected to hot water or autoclave treatment. For the hot water treatment the NaOH– CaCl_2 -heat-treated Ti metal was soaked in 10 ml of ultrapure water at 80 °C for 24 h. For the autoclave treatment the NaOH– CaCl_2 -heat-treated Ti metal was placed in an autoclave (SP300F, Yamato Scientific, Japan) at 121 °C for 0.5 h. The treated-Ti metals were gently washed with ultrapure water and then dried at 40 °C.

2.2. Surface characterization

Surface morphological changes of the Ti metal due to each treatment were observed by field emission scanning electron

microscopy (FE-SEM) (S-4300, Hitachi, Japan). The surface compositions of the treated-Ti metals were measured by energy dispersive X-ray analysis (EDX) (EMAX-7000, Horiba, Japan). Three specimens were prepared for EDX measurement for each group and five points were analyzed for each specimen (total 15 points per group). Distributions of some elements near the surface of the treated-Ti metal were measured by Auger electron spectroscopy (AES) (PHI-670, ULVAC-PHI Inc., Japan). The surface phases of the treated-Ti metals were analyzed by thin film X-ray diffractometry (TF-XRD) (RINT2500, Rigaku Co., Japan) and Raman spectroscopy (LabRam HR800, Horiba Ltd., Japan).

The abrasion resistance of the surfaces of the treated-Ti metals were examined with a scratch tester (CSR-2000, Rhesca Co. Ltd., Japan). A diamond tip stylus 5 μm in diameter was used at a 200 g mm^{-1} spring rate to measure the critical scratch point of the surface layer. The amplitude, scratch speed and load rate were 100 μm , 10 $\mu\text{m s}^{-1}$ and 100 mN min^{-1} , respectively, based on JIS R-3255. The mean values of critical scratch load were calculated from the measured values at six points for each group. The number of Ca^{2+} ions released from the treated-Ti metal by hot water treatment was measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (SPS3100, SII NanoTechnology Inc., Japan).

2.3. Examination of apatite-forming ability

The apatite-forming abilities of Ti metals subjected to each treatment were examined in pH 7.40 SBF comprising 142.0 mM Na^+ , 5.0 mM K^+ , 1.5 mM Mg^{2+} , 2.5 mM Ca^{2+} , 147.8 mM Cl^- , 4.2 mM HCO_3^- , 1.0 mM HPO_4^{2-} and 0.5 mM SO_4^{2-} , approximately equal to those of human blood plasma at 36.5 °C. The SBF was prepared by dissolving reagent grade NaCl, NaHCO_3 , KCl, $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, CaCl_2 and Na_2SO_4 in ultrapure water, and buffering at pH 7.40 with tris(hydroxymethyl)aminomethane $[(\text{CH}_2\text{OH})_3\text{CNH}_2]$ and 1.0 M HCl aqueous solution at 36.5 °C [18]. After soaking in SBF for 1 day the samples were removed from the solution and washed with ultrapure water. The surfaces of the samples soaked in SBF were observed by FE-SEM.

2.4. Moisture resistance test

The treated-Ti metals were kept in a chamber that controlled the relative humidity at 95% at 80 °C for 1 week. They were soaked in SBF for 1 day and washed with ultrapure water. Their surfaces were observed by FE-SEM. Surface apatite formation was compared with that of the surfaces before being kept in the humidity chamber.

3. Results

Fig. 1 shows FE-SEM pictures of the Ti metal surfaces after various treatments. A fine network structure formed after NaOH treatment. This network structure was not essentially changed by subsequent treatments.

FE-SEM pictures of cross-sections of the Ti metal surface layers subjected to various treatments are shown in Fig. 2. A lath-like phase grew upward from the substrate after NaOH treatment and essentially did not change after subsequent treatments.

According to EDX analysis about 5 at.% of Na^+ ions were incorporated into the surface of the Ti metal after NaOH treatment, as shown in Table 1. These Na^+ ions were maintained even after heat treatment. The Na^+ ions incorporated into the Ti metal surface by the NaOH treatment were completely replaced by Ca^{2+} ions on subsequent CaCl_2 treatment. The number of Ca^{2+} ions was not changed by subsequent heat treatment, although it decreased slightly with

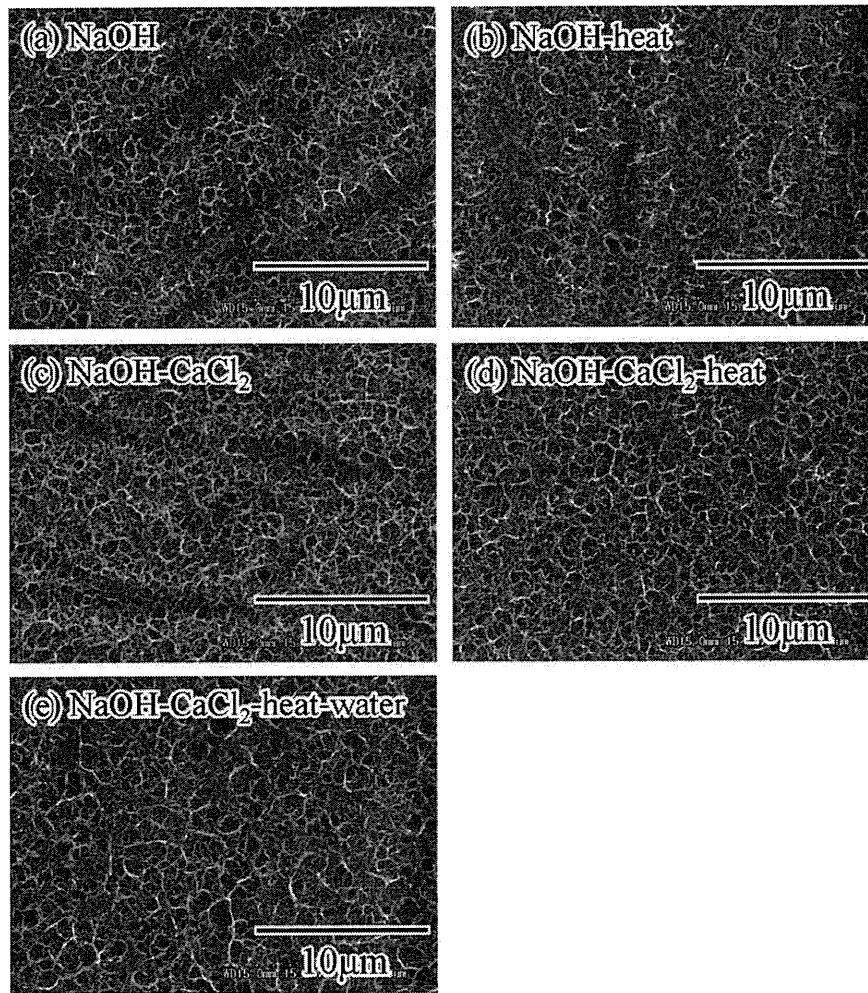


Fig. 1. FE-SEM photographs of the surfaces of Ti metals subjected to various treatments.

subsequent water treatment. The number of Ca^{2+} ions released into the water by hot water treatment was 0.15 ppm according to the ICP-AES measurements.

Depth profiles using Auger electron spectra of the Ti metal surfaces subjected to NaOH-heat and NaOH- CaCl_2 -heat-water treatments are shown in Fig. 3. The concentration of Na^+ ions incorporated into the Ti metal surface decreased with increasing depth within 1 μm . The concentration of Ca^{2+} ions incorporated into the surface layer of the NaOH- CaCl_2 -heat-water-treated Ti metal also decreased with increasing depth within about 1 μm . However, the calcium content at the top surface was a little lower than that at a depth of ~ 100 nm.

Figs. 4 and 5 show TF-XRD patterns and Raman spectra of the surface of Ti metals after various treatments. After the NaOH treatment sodium hydrogen titanate ($\text{Na}_x\text{H}_{2-x}\text{Ti}_3\text{O}_7 \cdot n\text{H}_2\text{O}$) [19–21] was detected for the first time. Subsequent heat treatment transformed the sodium hydrogen titanate into sodium titanate ($\text{Na}_2\text{Ti}_6\text{O}_{13}$) [19–21] and rutile. The sodium hydrogen titanate was speculated to be isomorphously transformed into calcium hydrogen titanate by CaCl_2 treatment after NaOH treatment, since the CaCl_2 treatment changed neither the XRD patterns nor Raman spectra. The heat treatment transformed the calcium hydrogen titanate into a low crystalline calcium titanate and rutile. Subsequent water treatment did not change these phases.

The scratch resistances of the surface layers formed on Ti metal by various treatments are shown in Fig. 6. Ti metals treated with NaOH solution or NaOH and CaCl_2 solutions had a low scratch

resistance, whereas those heat treated after chemical treatment had a remarkably high scratch resistance that did not decrease with subsequent water treatment. A significant difference in t -test was observed between the samples with heat treatment (NaOH-heat-, NaOH- CaCl_2 -heat- and NaOH- CaCl_2 -heat-water-treated Ti metals) and without heat treatment (NaOH- and NaOH- CaCl_2 -treated Ti metals).

Fig. 7 shows FE-SEM photographs of the surfaces of the Ti metals soaked in SBF for 1 day after the various treatments. The depositions formed on the treated-Ti metal surfaces were assigned as apatite by TF-XRD. The NaOH-treated Ti metal showed relatively low apatite-forming ability in SBF, however, the apatite-forming ability increased remarkably on subsequent heat treatment. The NaOH- CaCl_2 -treated Ti metal showed a slightly higher apatite-forming ability than the NaOH-treated Ti metal. Its apatite-forming ability decreased significantly with subsequent heat treatment, but increased remarkably after hot water or autoclave treatment following heat treatment. The Ca/P ratios of the apatite formed on the NaOH- CaCl_2 -heat-water-treated Ti metal was found to be 1.62 by EDX analysis. It was no different from that of the apatite formed on NaOH-heat-treated Ti metal.

It lost apatite-forming ability, also when it was stored at room temperature in air atmosphere for 2 month. However, it did not decrease apatite-forming ability when it was kept at 80 °C in air atmosphere for 1 week. Fig. 8 shows FE-SEM pictures of the surface of the NaOH-heat-treated and NaOH- CaCl_2 -heat-water-treated Ti metals which were soaked in SBF for 1 day after being kept at

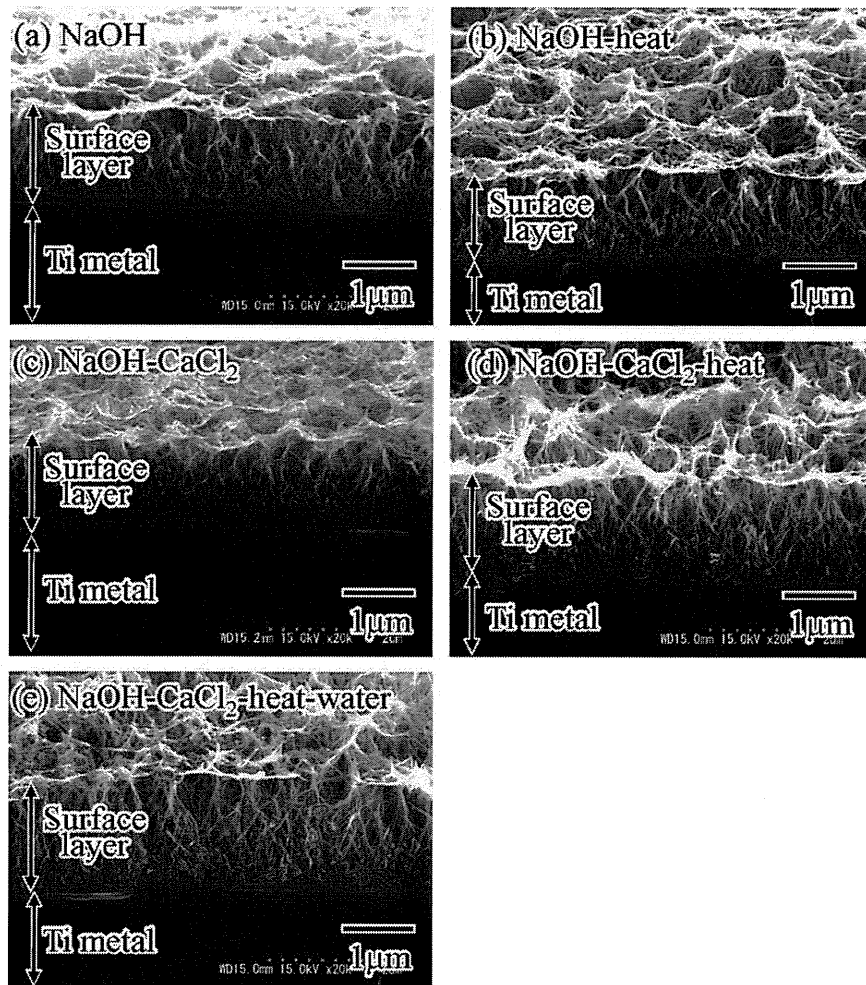


Fig. 2. FE-SEM photographs of cross-sections of Ti metals subjected to various treatments.

Table 1
Element concentration measured by EDX at surfaces of Ti metals subjected to various treatments.

Treatment	Element/at.%				
	Ti	O	C	Na	Ca
NaOH	26.5	62.5	5.8	5.3	0
(std. dev.)	(0.2)	(0.3)	(0.2)	(0.3)	(0)
NaOH-heat	25.9	65.1	3.5	5.6	0
(std. dev.)	(0.7)	(0.2)	(0.2)	(0.5)	(0)
NaOH–CaCl ₂	25.0	64.2	6.7	0	4.1
(std. dev.)	(0.2)	(0.3)	(0.3)	(0)	(0.1)
NaOH–CaCl ₂ -heat	25.9	65.0	4.9	0	4.1
(std. dev.)	(0.4)	(0.2)	(0.2)	(0)	(0.2)
NaOH–CaCl ₂ -heat-water	25.4	65.1	5.8	0	3.7
(std. dev.)	(0.3)	(0.5)	(0.2)	(0)	(0.1)

95% relative humidity at 80 °C for 1 week as a moisture resistance test. Apatite formation was not observed on the surface of the NaOH-heat-treated Ti metal after the moisture resistance test, whereas it was fully observed on NaOH–CaCl₂-heat-water-treated Ti metal even after the moisture resistance test.

About 30% of the Na⁺ ions at the surface of the NaOH-heat-treated Ti metal were lost because of the moisture resistance test, whereas the number of Ca²⁺ ions at the surface of NaOH–CaCl₂-heat-water-treated Ti metal did not change even after the moisture resistance test, as shown in Table 2. No structural change was observed in TF-XRD patterns of the NaOH-heat-treated and

NaOH–CaCl₂-heat-water-treated Ti metals after the moisture resistance test.

4. Discussion

It is apparent from the experimented results that a fairly large number of calcium ions were easily incorporated into the surface of Ti metals when first treated with a NaOH solution and then soaked in a CaCl₂ solution (Table 1 and Fig. 3). This is because the sodium hydrogen titanate (Na₂H_{2-x}Ti₃O₇·nH₂O) that formed on the Ti metal surface during the first NaOH treatment has a layered structure [19] and the Na⁺ ions in it were easily replaced by Ca²⁺ ions on CaCl₂ treatment (Fig. 4). However, the resultant Ti metal enriched with calcium ions on its surface showed only a slightly higher apatite-forming ability in SBF than NaOH-treated Ti metal (Fig. 7c) and had a low scratch resistance (Fig. 6). When heat treated at 600 °C, however, the resultant product had a high scratch resistance (Fig. 6), but showed extremely low apatite-forming ability (Fig. 7d). This might be attributed to the low mobility of the Ca²⁺ ions in the calcium titanate formed from calcium hydrogen titanate by heat treatment (Fig. 4). The apatite-forming ability of heat-treated Ti metal increased remarkably when it was subsequently treated with hot water or by autoclaving (Fig. 7e and f). This might be attributed to the increased mobility of Ca²⁺ ions in the calcium titanate due to the water or autoclave treatment. Table 1, Fig. 3 and ICP-AES measurements showed that a small number of Ca²⁺ ions at the surface of the treated-Ti metal were released

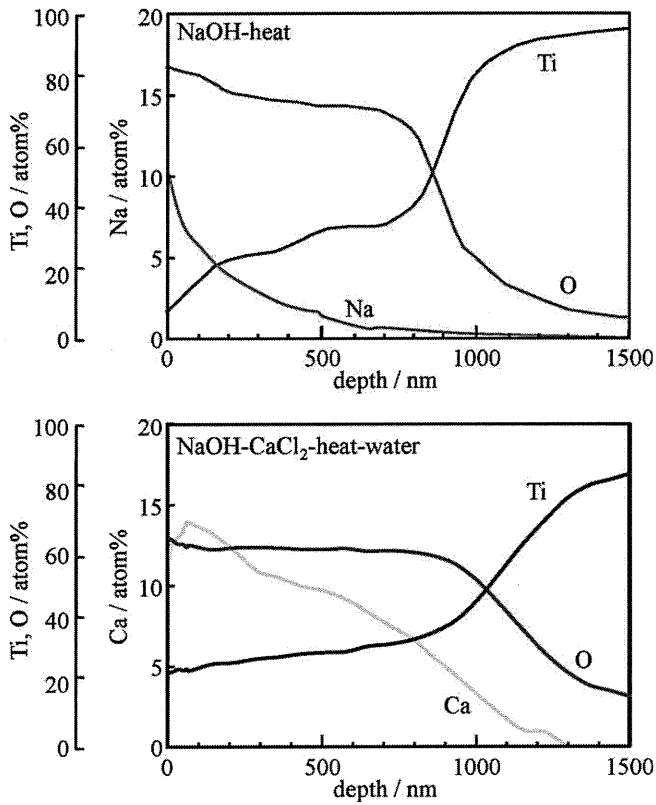


Fig. 3. AES depth profiles near the surfaces of NaOH-heat- and NaOH-CaCl₂-heat-water-treated Ti metals.

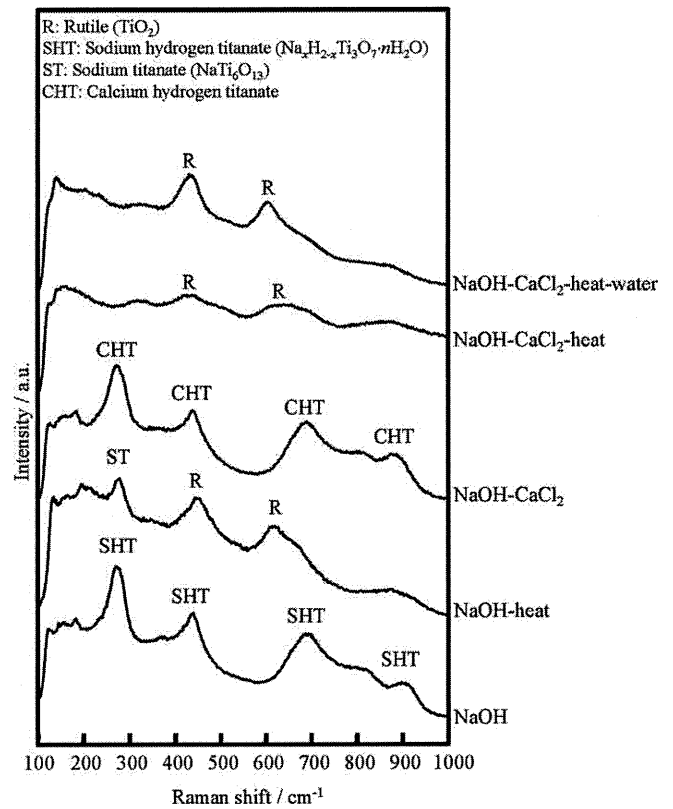


Fig. 5. Raman spectra of the surfaces of Ti metals subjected to various treatments.

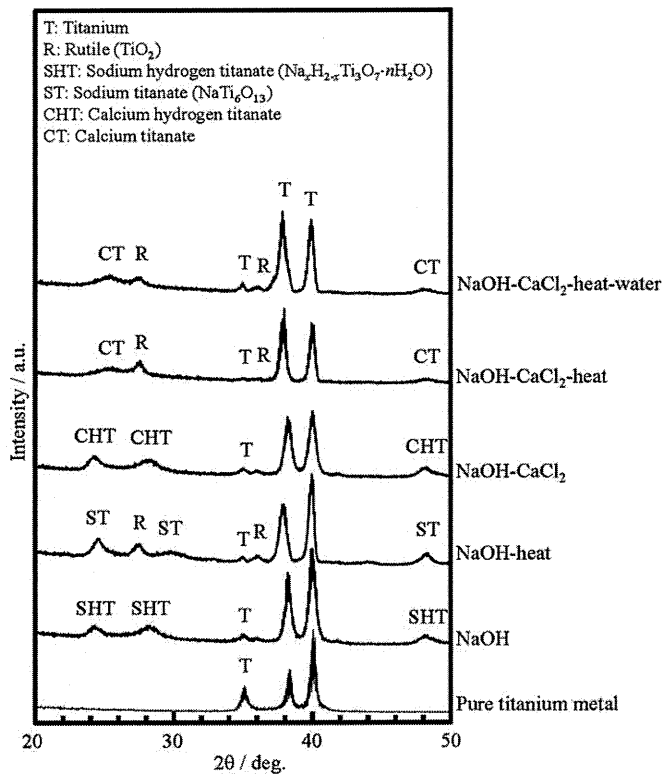


Fig. 4. TF-XRD patterns of the surfaces of Ti metals subjected to various treatments.

into the water on hot water treatment. This indicates that the Ca²⁺ ions in the calcium titanate were partially replaced with H₃O⁺ ions.

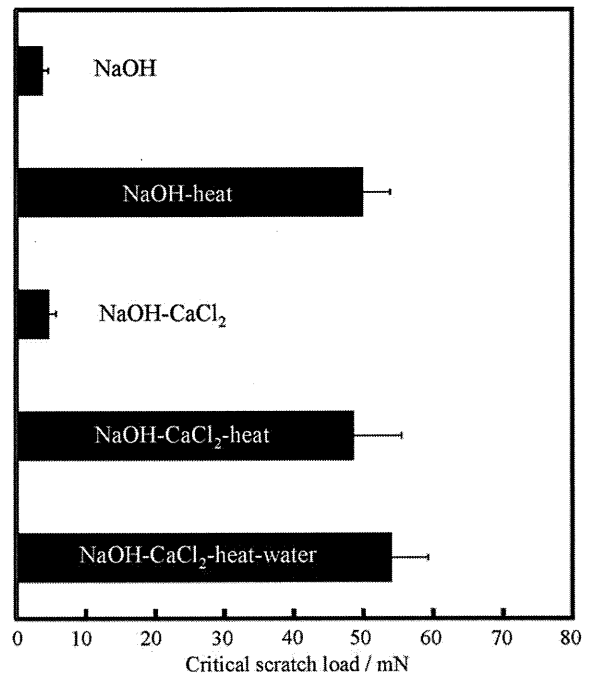


Fig. 6. Scratch resistance of the surface layers formed on Ti metals by the various treatments.

Usually monovalent cations such as H₃O⁺ ions have a higher mobility than divalent cations such as Ca²⁺ ions in an oxide crystal lattice [22]. Therefore, it is expected that Ca²⁺ ions could migrate easily in the crystal lattice when Ca²⁺ ions were partially replaced by H₃O⁺ ions. As a result, apatite is speculated to be formed on the surface of NaOH-CaCl₂-heat-water-treated Ti metal in SBF by the

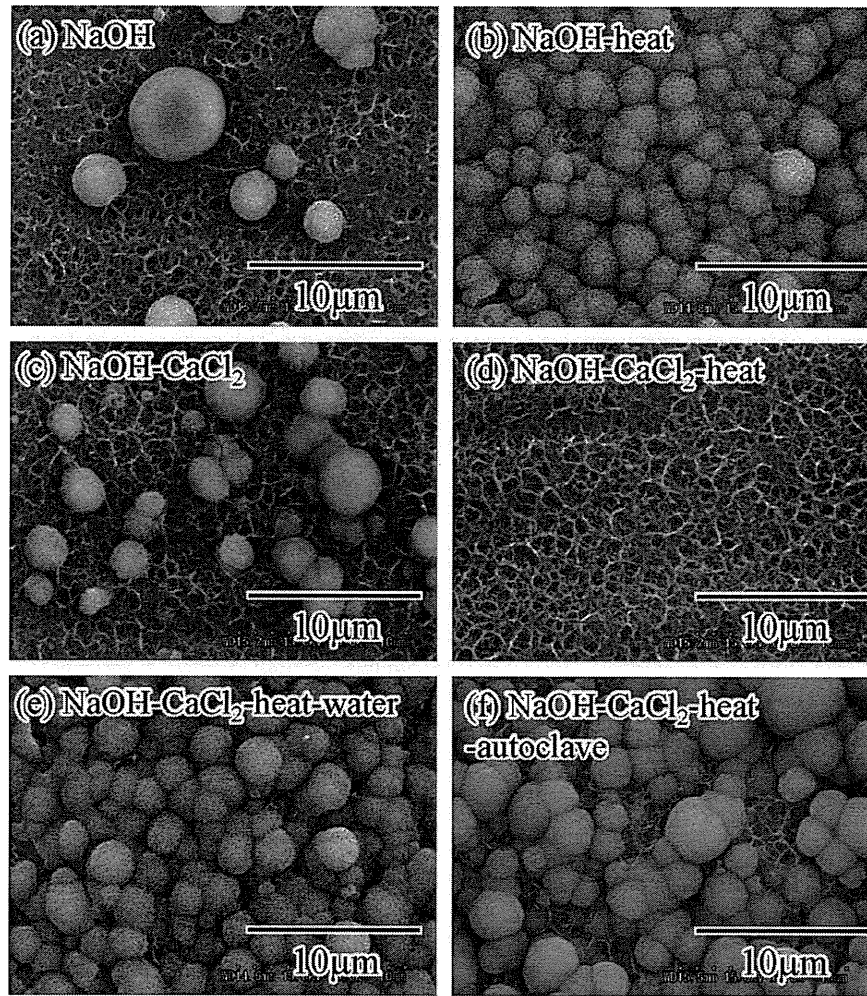


Fig. 7. FE-SEM pictures of the surfaces of Ti metals subjected to various treatments and soaked in SBF for 1 day.

following mechanism. In SBF Ca^{2+} ions in calcium titanate at the surface of the treated-Ti metal are released via exchange with H_3O^+ ions in the surrounding fluid. Ti–OH groups are formed on the surface of the Ti metal, similarly to Na^+ ions in sodium titanate at the surface of the NaOH-heat-treated Ti metal [23]. The released Ca^{2+} ions increase the ionic activity product of the apatite, as well as pH of the surrounding SBF. The Ti–OH groups formed are negatively charged and combine with positively charged Ca^{2+} ions in the SBF. As Ca^{2+} ions accumulate, the surface of the Ti metal is positively charged and combines with negatively charged phosphate ions, forming an amorphous calcium phosphate. This calcium phosphate is eventually transformed into crystalline bone-like apatite.

The result of the autoclave treatment showed that NaOH– CaCl_2 -heat-treated Ti metal can be supplied for use in medical devices without a final hot water treatment, since it has a high apatite-forming ability if it is sterilized using an autoclave before implantation. The apatite-forming ability of Ti metals treated with hot water and in an autoclave were, however, almost equal and barely that of the NaOH- and heat-treated Ti metal, despite the expectations described in Section 1. This might be attributed to a low mobility of Ca^{2+} ions compared with Na^+ ions in the titanates. Because of this low mobility of Ca^{2+} ions, the apatite-forming ability of NaOH– CaCl_2 -heat-water-treated Ti metal did not decrease, even after being kept in a humid environment (Fig. 8).

It has been reported for various kinds of materials able to form bone-like apatite on their surface in SBF also form apatite in the

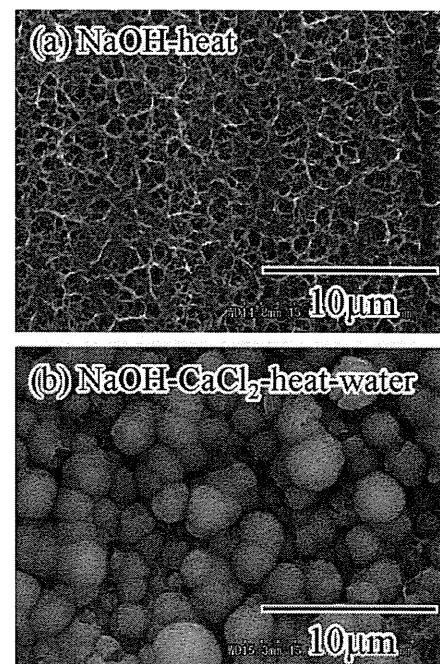


Fig. 8. FE-SEM pictures of the surfaces of Ti metals subjected to NaOH-heat and NaOH– CaCl_2 -heat-water treatments and soaked in SBF for 1 day after being kept at 95% relative humidity at 80 °C for 1 week.

Table 2

Element concentration measured by EDX at surfaces Ti metals subjected to various treatments before and after kept in humid environment.

Sample	Element/at.%				
	Ti	O	C	Na	Ca
NaOH-heat					
As-prepared	25.9	65.1	3.5	5.6	0
(std. dev.)	(0.7)	(0.2)	(0.2)	(0.5)	(0)
After kept in humid environment	26.3	66.1	3.8	3.8	0
(std. dev.)	(0.8)	(0.6)	(0.1)	(0.2)	(0)
NaOH–CaCl ₂ -heat-water					
As-prepared	25.4	65.1	5.8	0	3.7
(std. dev.)	(0.3)	(0.5)	(0.2)	(0)	(0.1)
After kept in humid environment	25.0	64.8	6.5	0	3.7
(std. dev.)	(0.3)	(0.2)	(0.4)	(0)	(0.1)

living body. Thus, the prepared Ti metal is expected to show a high apatite-forming ability even in the living body and to bond to living bone via the apatite layer. This makes it a bioactive metal useful for various kinds of medical devices.

5. Conclusion

A large number of Ca²⁺ ions were incorporated into Ti metal surfaces by NaOH and CaCl₂ treatment. Although the scratch resistance of the chemically treated surface layer was low, it increased remarkably with subsequent heat treatment. Although heat treatment took away the apatite-forming ability of the surface of the NaOH–CaCl₂-treated Ti metal, subsequent hot water or autoclave treatment increased the apatite-forming ability remarkably. The apatite-forming ability of the NaOH–CaCl₂ heat-water-treated Ti metal did not decrease, even when it was kept in a highly humid environment. Therefore, CaCl₂ treatment and the subsequent treatments of the NaOH-treated Ti metal were effective in producing high apatite-forming ability, high scratch resistance and high moisture resistance on the surface of the Ti metal.

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Apatite-forming ability of Ti–15Zr–4Nb–4Ta alloy induced by calcium solution treatment

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Received: 25 June 2009 / Accepted: 5 October 2009 / Published online: 20 October 2009
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Abstract Ti–15Zr–4Nb–4Ta alloy free from cytotoxic elements shows high mechanical strength and high corrosion resistance. However, simple NaOH and heat treatments cannot induce its ability to form apatite in the body environment. In the present study, this alloy was found to exhibit high apatite-forming ability when it was treated with NaOH and CaCl₂ solutions, and then subjected to heat and hot water treatments to form calcium titanate, rutile, and anatase on its surface. Its high apatite-forming ability was maintained even in 95% relative humidity at 80°C after 1 week. The surface layer of the treated alloy had scratch resistance high enough for handling hard surgical devices. Thus, the treated alloy is believed to be useful for orthopedic and dental implants.

1 Introduction

Titanium (Ti) alloys such as Ti–6Al–4V and Ti–6Al–7Nb have been widely used as orthopedic implants because of their high mechanical strengths and good biocompatibilities. Recently, new kinds of titanium-based alloys excluding elements suspected of cytotoxicity, such as vanadium (V) and aluminum (Al) [1–3], are being developed [4–9]. Ti–15Zr–4Nb–4Ta alloy is one such alloy, and it shows

high mechanical strength, relatively low elastic modulus, and better corrosion resistance compared with the Ti–6Al–4V alloy [5, 6]. However, this alloy does not bond to living bone.

It has been reported that simple chemical NaOH and heat treatments can provide bone-bonding ability in pure Ti metal [10–12] and its alloys such as Ti–6Al–4V, Ti–15Mo–5Zr–3Al, and Ti–6Al–2Nb–1Ta [13–15]. Because of their convenience and effectiveness, these kinds of chemical and heat treatments were applied to a porous Ti surface layer of an artificial total hip joint made of a Ti–6Al–2Nb–1Ta alloy. This bioactive artificial hip joint has been clinically used in Japan since 2007. However, these treatments are not effective for inducing bioactivity in Ti–15Zr–4Nb–4Ta alloys.

In the present study, chemical and heat treatments effective for inducing bioactivity in Ti–15Zr–4Nb–4Ta alloys were investigated. Bioactivity was evaluated by the apatite-forming ability in a simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma [16, 17].

2 Materials and methods

2.1 Surface treatments

The Ti–15Zr–4Nb–4Ta alloy (Kobe Steel, Ltd., Ti: Bal., Zr: 14.51, Nb: 3.83, Ta: 3.94, Pd: 0.16, O: 0.25 mass%) was cut into rectangular plates 10 × 10 × 1 mm³ in size, abraded with #400 diamond plates, and washed with acetone, 2-propanol, and ultrapure water in an ultrasonic cleaner for 30 min each, and dried at 40°C. The alloy samples were soaked in 5 ml of a 5 M NaOH aqueous solution at 60°C for 24 h. After removal from the solution,

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they were gently rinsed with ultrapure water for 30 s and dried at 40°C. The treated alloy samples were subsequently soaked in 10 ml of 100 mM CaCl₂ solution at 40°C for 24 h, and washed and dried in a similar manner. Then, they were heated to 600°C at a rate of 5°C/min and kept for 1 h, followed by natural cooling in an electrical furnace. After the heat treatment, they were soaked in 10 ml of ultrapure water at 80°C for 24 h, and then washed and dried.

2.2 Surface analyses

The surfaces of the alloy samples subjected to the chemical and heat treatments were analyzed by field emission scanning electron microscopy (FE-SEM: S-4300, Hitachi Co., Tokyo, Japan) equipped with energy dispersive X-ray (EDX: EMAX-7000, HORIBA Ltd., Kyoto, Japan) spectroscopy, Auger electron spectroscopy (AES: PHI-670, ULVAC-PHI Inc., Kanagawa, Japan), thin-film X-ray diffractometry (TF-XRD: RINT-2500, Rigaku Co., Tokyo, Japan), and Fourier transform confocal laser Raman spectroscopy (FT-Raman: LabRAM HR800, HORIBA Jobin-Yvon, France). In the FE-SEM and EDX analyses, accelerating voltages of 15 and 5 kV, respectively, were selected. In the TF-XRD measurement, a Cu K α X-ray source was used at 50 kV and 200 mA, with 0.01° step widths, 1 s/step scans, and 1° glancing angles against the incident beam. In the FT-Raman measurements, an argon (Ar) laser with a wavelength of 514.5 nm was used as a laser source.

Cross-sections of the alloy were also observed under FE-SEM with the same conditions as described above.

The scratch resistance of the surface layer formed on the alloy samples by the chemical and heat treatments was measured using a thin-film scratch tester (CSR-2000, Rhesca Co. Ltd., Tokyo, Japan), using a stylus 5 μ m in diameter with a spring constant of 200 g/mm. Based on the standard JIS R-3255, the amplitude, scratch speed, and loading rates were 100 μ m, 10 μ m/s, and 100 mN/min, respectively.

2.3 Apatite formation in SBF

The alloy samples subjected to the chemical and heat treatments were soaked in 24 ml of SBF with ion concentrations (Na⁺ 142.0, K⁺ 5.0, Ca²⁺ 2.5, Mg²⁺ 1.5, Cl⁻ 147.8, HCO₃⁻ 4.2, HPO₄²⁻ 1.0, and SO₄²⁻ 0.5 mM) nearly equal to those of human blood plasma at 36.5°C. The SBF was prepared by dissolving reagent grade NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂, and Na₂SO₄ (Nacalai Tesque Inc., Kyoto, Japan) in ultrapure water, and buffered at pH = 7.4 with tri-hydroxymethylaminomethane (CH₂OH)₃CNH₂ and 1 M HCl (Nacalai Tesque Inc., Kyoto, Japan) at 36.5°C [16, 17]. After soaking in SBF for 3 d, the samples were removed, gently rinsed with ultrapure water

for 30 s, and dried at 40°C. Apatite formation on their surfaces was examined by TF-XRD, FE-SEM, and EDX. To examine the stability of the apatite-forming ability, the treated alloy samples were kept for one week in an incubator with a relative humidity of 95% at 80°C. After removal from the incubator, their apatite-forming abilities were examined by soaking in SBF for 3 d.

3 Results

3.1 Surface structures

Table 1 shows the EDX results for the surfaces of the untreated alloy samples and those subjected to the NaOH, CaCl₂, heat, and water treatments. The table shows that about 3.8 at.% of sodium (Na) was incorporated into the surface of the alloy. By the second CaCl₂ treatment, the incorporated Na was completely replaced with calcium (Ca), and a little more Ca was incorporated into the surface. The amount of the incorporated Ca was not changed by the heat treatment, but was decreased a little by the final water treatment.

Figure 1 shows an AES depth profile of the surface of the Ti-15Zr-4Nb-4Ta alloy subjected to the NaOH, CaCl₂, heat, and water treatments. The figure shows that zirconium

Table 1 Chemical compositions of surface layers of Ti-15Zr-4Ta-4Nb alloy untreated and subjected to NaOH, CaCl₂, heat and water treatments, which were analyzed by EDX

	Elements (at.%)							
	O	Ti	Na	Ca	Zr	Nb	Ta	Pd
Untreated	7.2	82.8	0	0	7.1	2.0	0.7	0.1
NaOH	58.1	36.2	3.8	0	1.0	0.6	0.2	0.0
NaOH-CaCl ₂	58.4	34.5	0	5.6	0.8	0.5	0.3	0.1
NaOH-CaCl ₂ -heat	58.2	34.9	0	5.4	0.7	0.5	0.2	0.0
NaOH-CaCl ₂ -heat-water	58.6	35.6	0	4.4	0.7	0.5	0.2	0.0

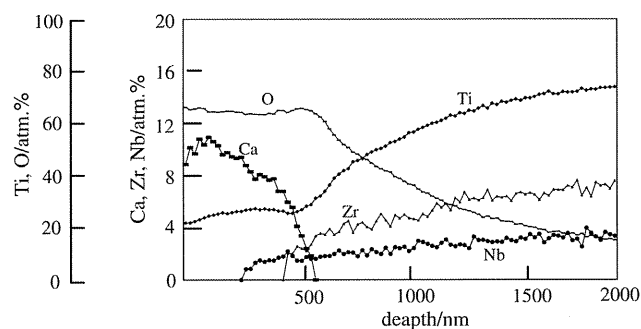


Fig. 1 AES depth profiles of the surface of Ti-15Zr-4Nb-4Ta alloy subjected to NaOH, CaCl₂, heat and water treatments