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Nanostructured positively charged bioactive TiO_2 layer formed on Ti metal by NaOH, acid and heat treatments

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Abstract Nanometer-scale roughness was generated on the surface of titanium (Ti) metal by NaOH treatment and remained after subsequent acid treatment with HCl, HNO_3 or H_2SO_4 solution, as long as the acid concentration was not high. It also remained after heat treatment. Sodium hydrogen titanate produced by NaOH treatment was transformed into hydrogen titanate after subsequent acid treatment as long as the acid concentration was not high. The hydrogen titanate was then transformed into titanium oxide (TiO_2) of anatase and rutile by heat treatment. Treated Ti metals exhibited high apatite-forming abilities in a simulated body fluid especially when the acid concentration was greater than 10 mM, irrespective of the type of acid solutions used. This high apatite-forming ability was maintained in humid environments for long periods. The high apatite-forming ability was attributed to the positive surface charge that formed on the TiO_2 layer and not to the surface roughness or a specific crystalline phase. This positively charged TiO_2 induced apatite formation by first selectively adsorbing negatively charged phosphate ions followed by positively charged calcium ions. Apatite formation is expected on the surfaces of such treated Ti metals after short periods, even in living systems. The bonding of metal to living bone is also expected to take place through this apatite layer.

1 Introduction

Various surface treatment methods have been employed in an attempt to induce the bone-bonding property of titanium (Ti) metal and its alloys [1–14]. Of the treatment methods considered, the formation of sodium titanate on Ti metal by treating with both NaOH and heat has been shown to be effective for inducing apatite formation on Ti metal in the human body and causing Ti metal to bond to living bone through the apatite layer [15–18]. This method was applied to a porous Ti metal layer of an artificial hip joint, and the resulting bone bonding, i.e., a bioactive hip joint, has been clinically used in Japan since 2007 [19]. Apatite formation on NaOH- and heat-treated Ti metal in the living body was attributed to the negative surface charge of sodium titanate.

In contrast, it was recently shown that combining acid and heat treatment to form TiO_2 on Ti metal effectively induces apatite formation in the body environment and causes Ti metal to bond to living bone through the apatite layer [20]. The apatite formation in this case was attributed to the positive surface charge of TiO_2 [20].

Treatment with NaOH generates a bioactive surface with nanometer-scale roughness having a high specific surface area, but the sodium titanate formed by this treatment may have an undesirable effect on living cells in the narrow spaces of the porous material by the release Na^+ ions. In contrast, treatment with acid generates a bioactive surface with micrometer-scale roughness having a low specific surface area, but the TiO_2 formed by this treatment does not release ions that are liable to have adverse effects on living cells. Recently, it was shown that when Ti metal is subjected to HCl and heat treatment [21] subsequent to NaOH treatment, the bioactive TiO_2 layer that was formed had nanometer-scale roughness with a high specific surface area and releases no ions. In addition, porous Ti metal that

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had been subjected to these treatments exhibited higher values of osteoconductivity and osteoinductivity than that subjected to simple NaOH and heat treatment [22–25].

In this study, we investigated the type of acid treatment that would be effective for inducing high apatite-forming ability of TiO_2 formed on Ti metal. Treatment with NaOH, acid, and heat were investigated. Factors governing their apatite-forming abilities were discussed in terms of their surface structure and properties.

Several articles that investigate the apatite-forming ability of TiO_2 that is formed on Ti metal by various treatment methods have been published [26–36]. However, the principal factors governing apatite formation on TiO_2 are not yet clearly understood.

2 Materials and methods

2.1 Preparation of the samples

Commercially pure Ti metal (Kobe Steel, Ltd, Japan) was cut into rectangular samples with dimensions of 10 × 10 × 1 mm^3, abraded with a #400 diamond plate, washed with acetone, 2-propanol, and ultra pure water for 30 min each in an ultrasonic cleaner, and then dried overnight in an oven at 40°C. Each sample was soaked in 5 ml of a 5 M NaOH solution at 60°C in an oil bath, shaken at 120 strokes/min for 24 h, and then gently washed with ultra pure water. Subsequently, the samples were soaked in 10 ml of an HCl, HNO_3, or H_2SO_4 solution with concentrations ranging from 0.5 to 100 mM at 40°C in an oil bath, shaken at 120 strokes/min for 24 h, and then gently washed with ultra pure water and dried overnight in an oven at 40°C. The samples were heated to 600°C at a rate of 5°C/min in an Fe–Cr electric furnace, maintained at this temperature for 1 h, and then cooled naturally to room temperature in the furnace.

2.2 Surface analysis of the treated Ti metal

The surface of the Ti metal treated as described above was analyzed using thin film X-ray diffraction (TF-XRD, RINT-2500, Rigaku Co., Japan). The X-ray source used was CuKα, and the angle of the incident beam was set to 1° against the sample surface. The same surface was coated with a Pt/Pd film and observed under a field emission scanning electron microscope (FE-SEM, Hitachi S-4300, Hitachi, Japan).

Ti metal plates with dimensions of 13 × 33 × 1 mm^3 were used to measure the zeta potential, and in the NaOH and acid treatments of the samples, the volumes of the NaOH and acid solutions used were increased to 20 and 30 ml, respectively. The treated Ti metal plates were electrically grounded to discharge any stray charges, and were immediately set in the zeta potential and particle size analyzer (ELS-Z1, Otsuka Electronics Co., Japan) using a glass cell for the plate sample. The zeta potential of the samples was measured under an applied voltage of 40 V in a 10 mM NaCl solution dispersing monitor particles of polystyrene latex particles (diameter = 500 nm) coated with hydroxyl propyl cellulose. Five samples were measured for each experimental condition, and the average value was used in our analysis.

2.3 Examination of the apatite-forming ability in a simulated body fluid (SBF)

The Ti metals treated as described above were soaked in 30 ml of an acellular SBF at 36.5°C with ion concentrations ($Na^+ = 142.0$, $K^+ = 5.0$, $Mg^{2+} = 1.5$, $Ca^{2+} = 2.5$, $Cl^- = 147.8$, $HCO_3^- = 4.2$, $HPO_4^{2-} = 1.0$, and $SO_4^{2-} = 0.5$ mM) nearly equal to those of human blood plasma. The SBF was prepared by dissolving reagent-grade NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$, and Na_2SO_4 (Nacalai Tesque Inc., Japan) in ultra pure water, and buffering at pH = 7.40 using tris (hydroxymethyl) aminomethane [$(CH_2OH)_3CNH_2$] and 1 M HCl (Nacalai Tesque Inc.) [37].

After soaking for periods of 1 min and 12 h, the samples were removed from the SBF, gently washed with ultra pure water for 5 min and dried at 40°C in an oven. The sample's surface was analyzed using X-ray photoelectron spectroscopy (XPS, ESCA-3300KM, Shimadzu Co., Japan) using MgKα radiation ($\lambda = 9.8903$ Å) 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 of the substrate. The binding energy of the measured spectra was calibrated by reference to the C_{1S} peak of the surfactant's CH_2 group on the substrate occurring at 284.6 eV.

After soaking in the SBF for 1 day, the surface was analyzed for apatite formation using TF-XRD and FE-SEM. To examine the stability of the apatite-forming ability in a humid environment, the NaOH, acid, and heat-treated samples were kept at a relative humidity of 95% at 80°C for 1 week, and the apatite formed on their surface in the SBF was examined using FE-SEM.

3 Results

3.1 Surface structure of the treated Ti metals

Figure 1 shows FE-SEM photographs of the surface of Ti metals subjected to various acid treatments after an NaOH treatment, compared with the surface of untreated Ti metal and that treated with an NaOH solution. The treatments shown in Fig. 1 are designated according to the classifications shown in Table 1. A fine network structure of nanometer-scale was generated on the surface of the Ti metal by an initial NaOH treatment, and this remained essentially unchanged after a subsequent acid treatment, as long as the acid concentration was not high. The network structure was completely dissolved by treatment with a concentrated acid solution. The maximum concentration of the acid solution in which the fine network structure remained was 50 mM for the HCl and HNO_3 solutions, and 10 mM for the H_2SO_4 solution.

Figure 2 shows FE-SEM photographs of the surface of Ti metals subjected to a heat treatment after an NaOH and acid treatment. The surface structure formed on the Ti metals by the NaOH and acid treatments remained essentially unchanged after the heat treatment.

Figures 3 and 4 show the TF-XRD patterns of the surface of Ti metals subjected to NaOH and acid treatments, and those of Ti metals subjected to a subsequent heat treatment, respectively. From Fig. 3, it can be seen that a layer of sodium hydrogen titanate ($Na_xH_{2-x}Ti_3O_7$, $0 < x < 2$) had formed on the surface of Ti metal after the initial NaOH treatment [38], and that this was converted to

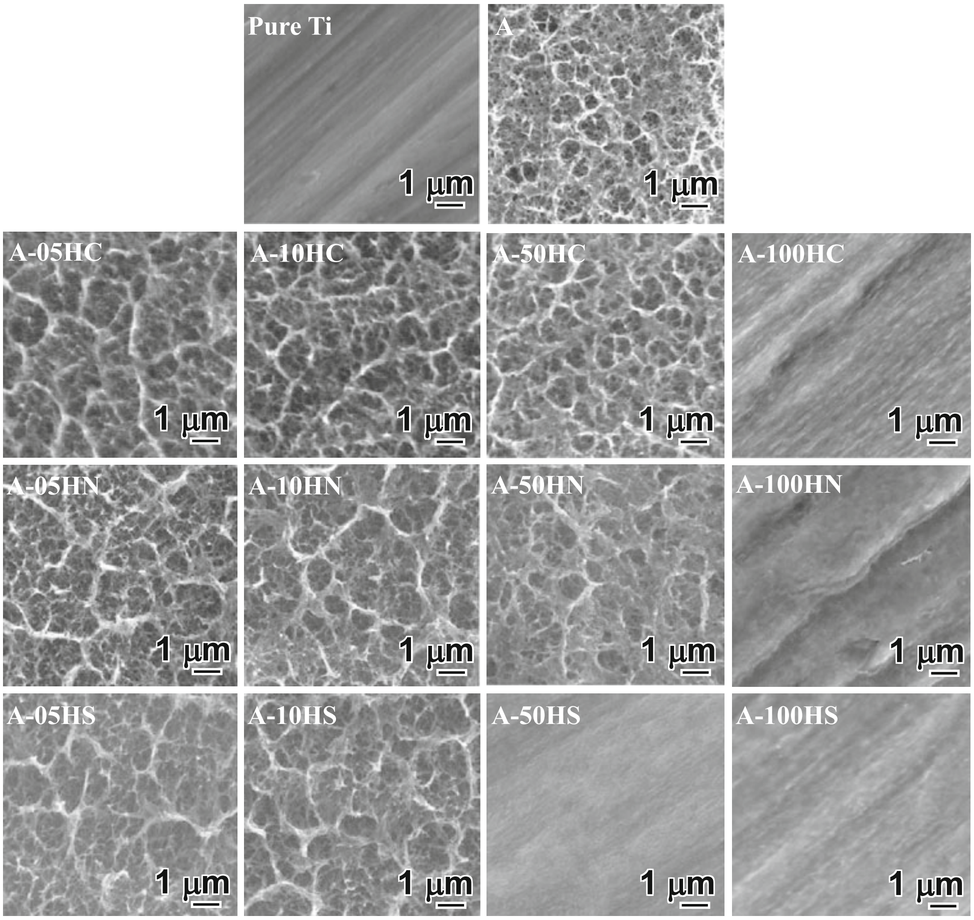


Fig. 1 FE-SEM photographs of surfaces of Ti metals subjected to various acid treatments after NaOH treatment, in comparison with those of Ti metal untreated and as-treated with NaOH. Explanation of notation is given in Table 1

Table 1 Notation of treatments

Notation	Treatments
A	Only NaOH treatment
A-05, 10, 50, or 100HC	A + 0.5, 10, 50 or 100 mM HCl treatments
A-05, 10, 50, or 100HC–H	A + 0.5, 10, 50 or 100 mM HCl + heat treatments
A-05, 10, 50, or 100HN	A + 0.5, 10, 50 or 100 mM HNO_3 treatments
A-05, 10, 50, or 100HN–H	A + 0.5, 10, 50 or 100 mM HNO_3 + heat treatments
A-05, 10, 50, or 100HS	A + 0.5, 10, 50 or 100 mM H_2SO_4 treatments
A-05, 10, 50, or 100HS–H	A + 0.5, 10, 50 or 100 mM H_2SO_4 + heat treatments

Fig. 2 FE-SEM photographs of surfaces of Ti metal subjected to the heat treatment after the acid and NaOH treatments, in comparison with those of Ti metal subjected to heat treatment without and after the NaOH treatment

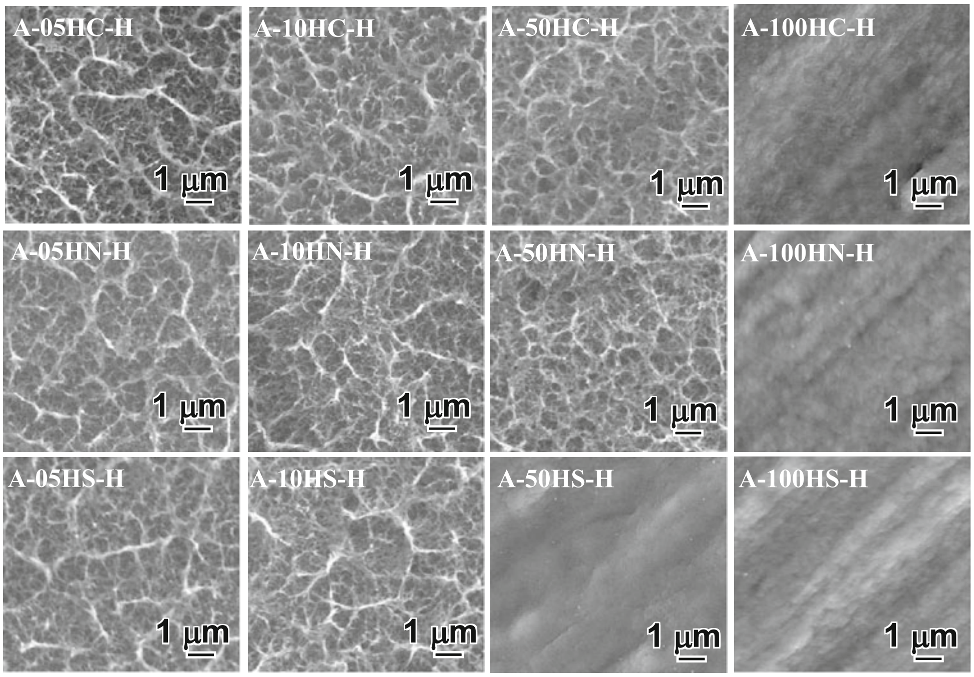
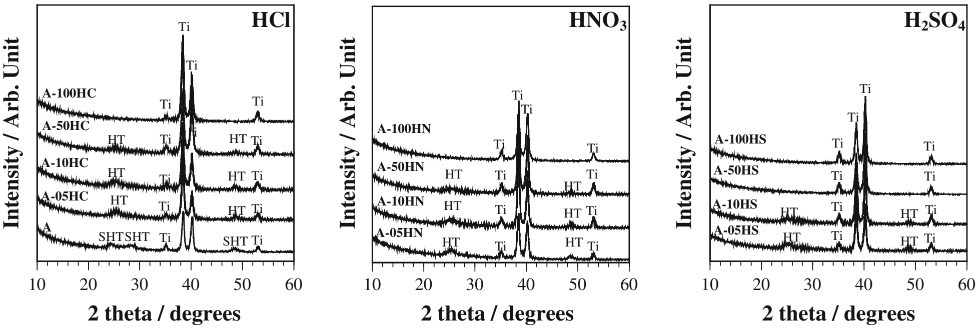


Fig. 3 TF-XRD of surfaces of Ti metal subjected to different chemical treatments. *Ti* α Ti, *HT* Hydrogen titanate, *SHT* Sodium hydrogen titanate



hydrogen titanate ($H_2Ti_3O_7$) [39] after a subsequent acid treatment, if the concentration of the acid solution was not high. After treatment with concentrated acid solutions, the sodium hydrogen titanate layer was completely dissolved. All the NaOH- and acid-treated Ti metals precipitated TiO_2 of anatase and/or rutile after the heat treatment (Fig. 4). The ratio of rutile to anatase increased with increasing concentration of the acid solution. Only the rutile phase was observed for the HCl and HNO_3 solutions with a concentration of 100 mM, and H_2SO_4 solutions with a concentration of 50 and 100 mM.

3.2 Apatite-forming ability of treated Ti metals in an SBF

Figures 5 and 6 show FE-SEM photographs of the surface of Ti metals soaked in an SBF for 1 day after different chemical treatments and a subsequent heat treatment, respectively. Spherical particles were observed on the surface, and these were identified as crystalline apatite from the TF-XRD data. Ti metals subjected to the chemical treatment alone did not form (or only formed a little) apatite on their surface in an SBF within a period of 1 day (Fig. 5). In contrast, all the Ti metals subjected to a heat treatment after a chemical treatment formed appreciable amounts of apatite on their surfaces in an SBF within 1 day (Fig. 6). The apatite-forming ability of the samples was high when the concentration of the acid solution used was greater than 10 mM.

Figure 7 shows FE-SEM photographs of the surface of Ti metals soaked in an SBF for 1 day after being kept at a relative humidity of 95% at 80°C for 1 week following NaOH, acid, and heat treatments. From Fig. 7, it can be seen that a high apatite-forming ability of such treated Ti metals was maintained, even in a humid environment.

3.3 Zeta potential of treated Ti metals

The zeta potentials of Ti metals treated with an acid solution after an NaOH treatment were not able to be

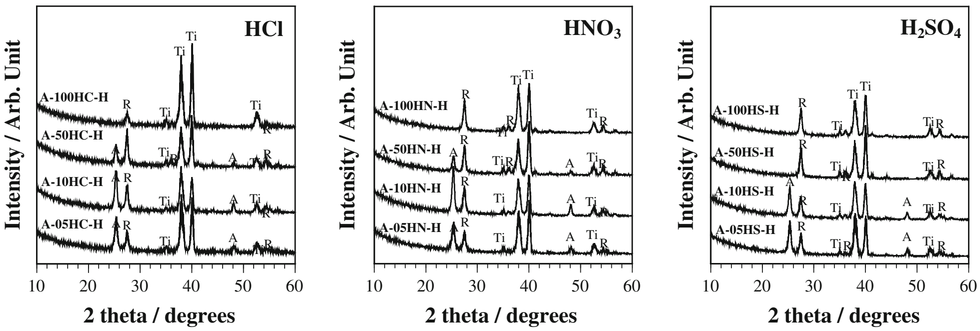


Fig. 4 TF-XRD of surfaces of Ti metal subjected to the heat treatment after different chemical treatments. *Ti* α Ti, *A* Anatase, *R* Rutile, *ST* Sodium titanate

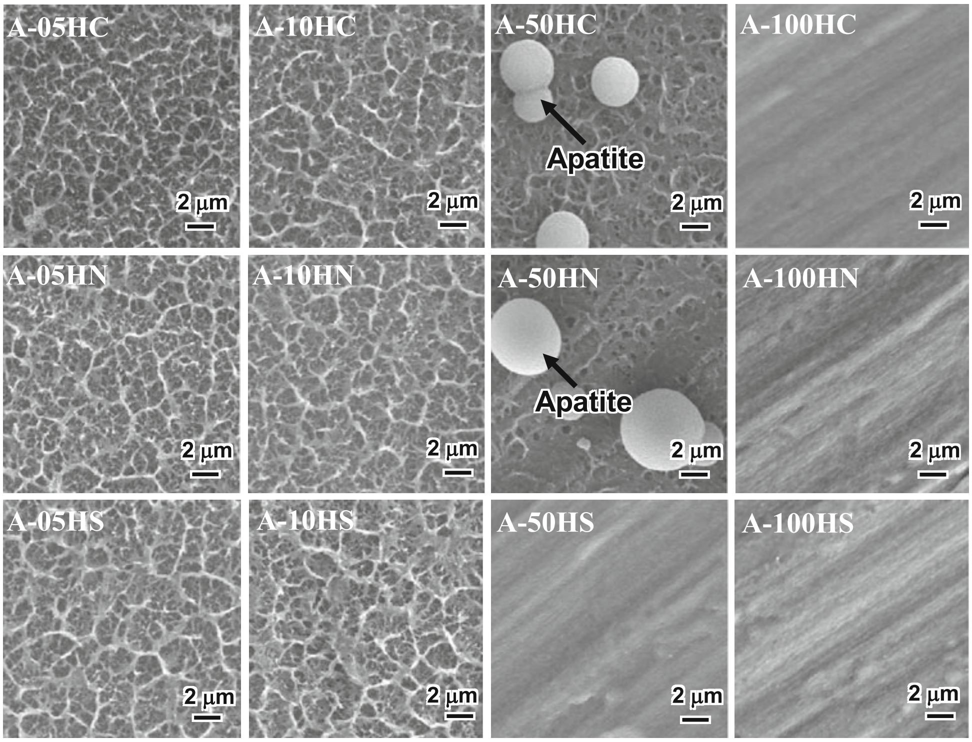


Fig. 5 FE-SEM photographs of surfaces of Ti metals soaked in SBF for 1 day after different chemical treatments

measured, as no insulating TiO_2 layer formed. This indicates that the zeta potential of these samples was almost zero. Figure 8 shows the zeta potentials of Ti metals subjected to a heat treatment after an NaOH and acid treatment. All the heat-treated samples showed a positive zeta potential, although the zeta potentials of Ti metals treated with 0.5 mM acid solutions were as low as 4 mV.

3.4 XPS spectra of treated Ti metals

Figure 9 shows the Ca_{2P} and P_{2P} XPS spectra of the surfaces of Ti metals soaked in an SBF for 1 min and 12 h after NaOH, acid, and heat treatments. From Fig. 9, it can be seen that all the treated Ti metals selectively adsorbed phosphate ions on their surface in an SBF within a period of 1 min, and later on, also adsorbed calcium ions to form calcium phosphate.

Figure 10 shows the Cl_{2P} and N_{1S} XPS spectra of the surfaces of Ti metal subjected to a heat treatment following an NaOH and 50 mM HCl or HNO_3 treatment. Also shown are the S_{2P} spectra of the surfaces of Ti metal treated with a 10 mM H_2SO_4 solution after an NaOH treatment after being subjected to a subsequent heat treatment and then kept at a relative humidity of 95% at 80°C for 1 week. From Fig. 10, it can be seen that chloride, nitrate, and sulfate ions were observed on the surface of Ti metals heat-treated after an HCl, HNO_3, and H_2SO_4 treatment following an NaOH treatment. Sulfate ions were detected on the surface of the samples before the heat treatment, and even after being kept in a humid environment for 1 week.

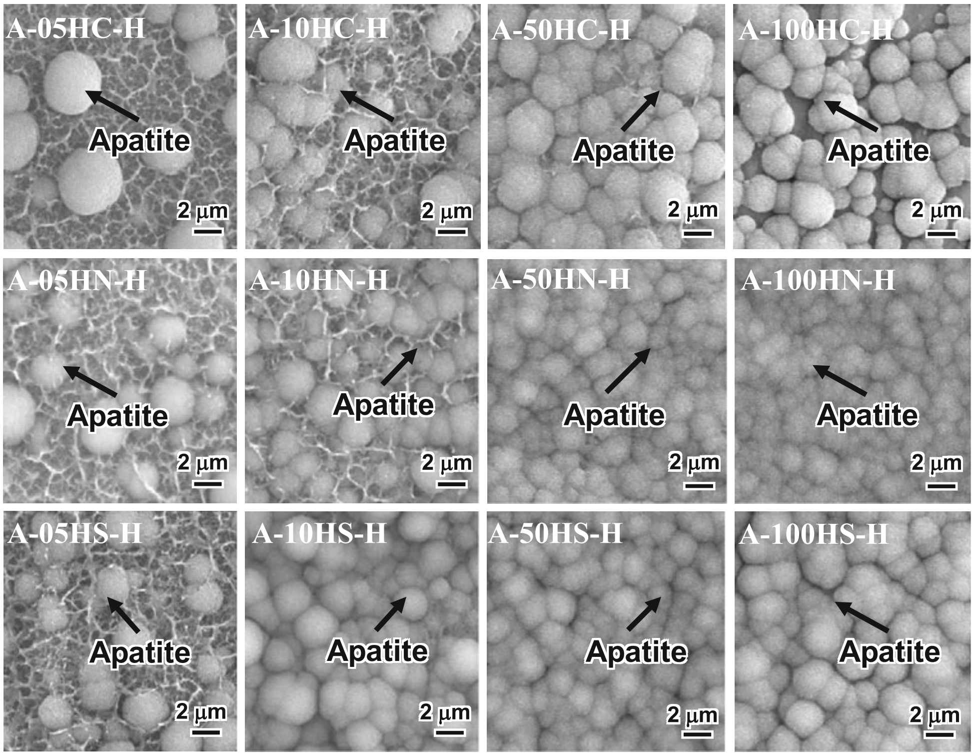


Fig. 6 FE-SEM photographs of surfaces of Ti metals soaked in SBF for 1 day after heat treatment following different chemical treatments

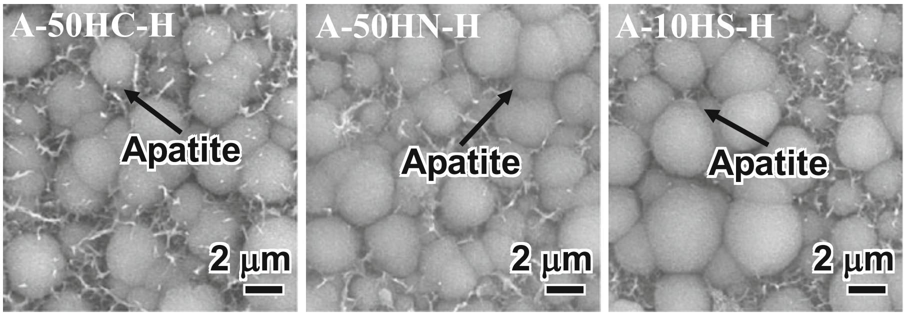


Fig. 7 FE-SEM photograph of surface of Ti metals soaked in SBF for 1 day, after kept in humid environment for 1 week following the NaOH, acid and heat treatments

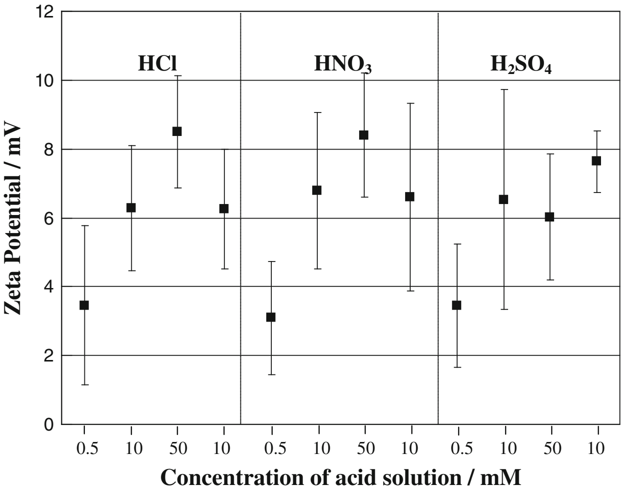


Fig. 8 Zeta potentials of surfaces of Ti metal plates after different chemical and heat treatments

4 Discussion

It is apparent from Figs. 1 and 2 that a nanometer-scale roughness is generated on the surface of Ti metals by an NaOH treatment and remained after the subsequent acid and heat treatments as long as the acid concentration is not high.

According to Figs. 3 and 4, sodium hydrogen titanate formed on Ti metals by an NaOH treatment is transformed into hydrogen titanate or completely dissolved by the subsequent acid treatment and then transformed into TiO_2 of anatase and/or rutile by the heat treatment.

It is apparent from Figs. 5 and 6 that the apatite-forming ability in an SBF of Ti metals subjected to an acid treatment after an NaOH treatment was very low before a heat treatment, but increased markedly after a heat treatment, irrespective of the type of acid solution used.

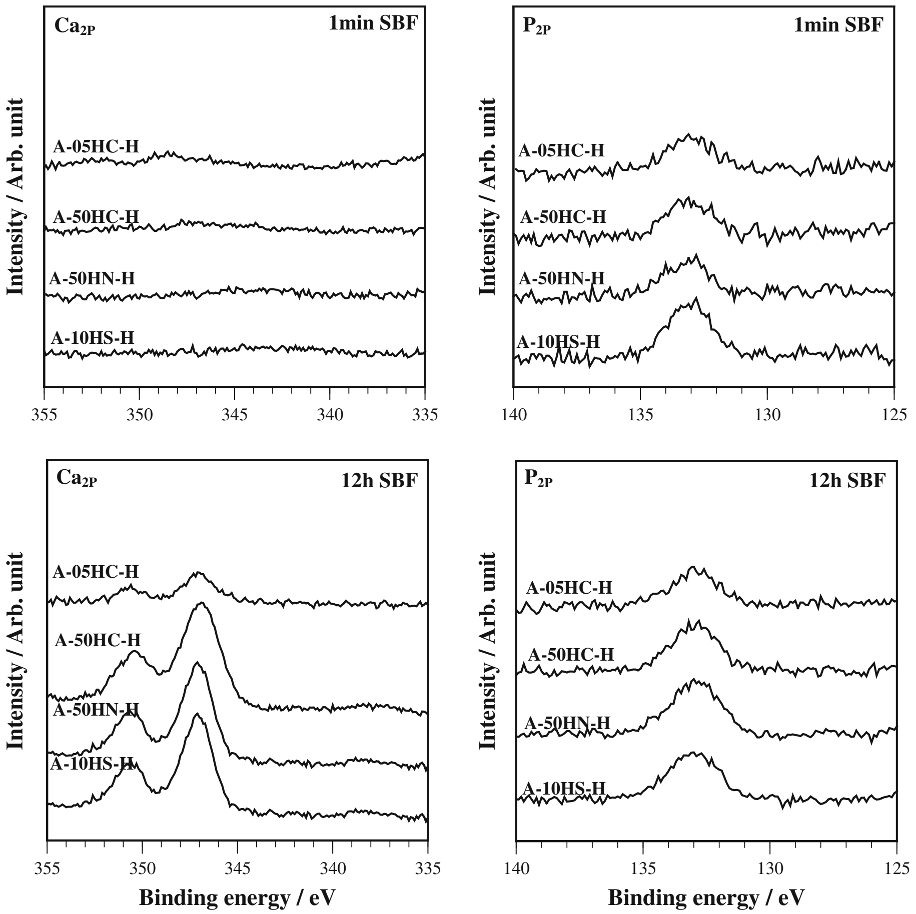
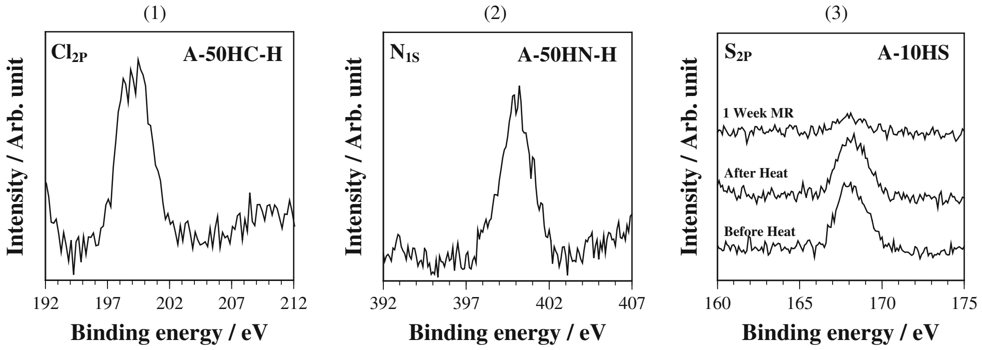


Fig. 9 XPS spectra of surfaces of Ti metals soaked in SBF for 1 min and 12 h after different chemical and heat treatments


(1)

(2)

(3)

Fig. 10 XPS spectra of surfaces of Ti metals subjected to various chemical and heat treatments. *1* after heat treatment following NaOH and 50 mM HCl treatment; *2* after heat treatment following NaOH and 50 mM HNO_3 treatment; *3* before and after heat treatment following the NaOH and 10 mM H_2SO_4 treatment, and after subsequent storage in 95% relative humidity at 80°C for 1 week

As described above, the surface roughness of Ti metal subjected to an acid treatment after an NaOH treatment did not change after a heat treatment. Therefore, the increase in the apatite-forming ability after the heat treatment cannot be attributed to the change in surface roughness.

As described above, the surface phase of the Ti metals subjected to an acid treatment after an NaOH treatment was either hydrogen titanate or titanium before the heat treatment, while it was TiO_2 of anatase and/or rutile after the heat treatment. This indicates that TiO_2 is responsible for the high apatite-forming ability of the heat-treated Ti metal. However, apatite-forming ability of the heat-treated Ti metal does not depend upon the type of TiO_2 formed. For example, Ti metal treated with a 10 mM H_2SO_4 solution showed an almost equal apatite-forming ability to that of sample treated with a 100 mM H_2SO_4 solution (see

Fig. 6), although the former sample had a larger amount of anatase precipitated than rutile, whereas the latter sample had precipitated only rutile (see Fig. 4). Therefore, the apatite-forming ability of the heat-treated Ti metal samples cannot be attributed to the specific crystalline phase of TiO_2 precipitated.

The zeta potential of Ti metals subjected to an acid solution after an NaOH treatment was almost zero before a heat treatment, as described above. According to Fig. 8, the zeta potential of Ti metals subjected to an acid treatment after an NaOH treatment showed positive values after the heat treatment, and high values were observed when the concentration of the acid solution was greater than 10 mM, irrespective of the type of acid solution used. Figures 5 and 6 show that the apatite-forming ability of Ti metals subjected to an acid treatment after an NaOH treatment was increased by the heat treatment and became high when the concentration of the acid solution was greater than 10 mM, irrespective of the type of acid solution used.

When Ti metals have a positively charged surface, then negatively charged phosphate ions can be selectively adsorbed on their surface first. As these phosphate ions accumulate, the surface becomes negatively charged. As a result, positively charged calcium ions can be adsorbed on the surface to produce apatite. This sequential adsorption of phosphate and calcium ions was confirmed in the XPS spectra of Ti metals heat-treated after various acid treatments shown in Fig. 9. From these observations, it can be concluded that the high apatite-forming ability of the Ti metals heat-treated after an acid treatment following an NaOH treatment can be attributed to their positive surface charge.

The reason why the Ti metals heat-treated after an acid treatment following an NaOH treatment had a positive surface charge can be interpreted in terms of the acid groups adsorbed on their surface after the acid treatment. It was confirmed by the XPS spectra shown in Fig. 10 that sulfate ions were adsorbed on Ti metal soaked in an H_2SO_4 solution after an NaOH treatment, and that these remained on the Ti metal surface, even after subsequent heat treatment. Chloride ions and nitrate ions were also confirmed to be adsorbed on the Ti metal heat-treated after treatment in an HCl and HNO_3 solution, as shown in Fig. 10. These acid groups can dissociate from the surface of the Ti metals in an SBF to give an acidic surface environment. TiO_2 has been reported to be positively charged in acidic aqueous solutions, and its positive charge increases with decreasing pH of the solution [40, 41]. The acid solutions used in this study had pH values that decreased with increasing acid concentration, as shown in Table 2. All the acid solutions used in this study had pH values lower than 2 when their concentration was greater than 10 mM.

Table 2 pH values of acid solutions used in the present study

Solution	pH			
	0.5 mM	10 mM	50 mM	100 mM
HCl	3.46	2.13	1.47	1.16
HNO_3	3.50	2.14	1.46	1.15
H_2SO_4	3.10	1.98	1.25	1.05

The acid groups adsorbed on the Ti metals heat-treated after an acid treatment remained, even after the Ti metals were stored in a humid environment at high temperature, as shown in Fig. 10. Therefore, their high apatite-forming ability was maintained, even after storage in a humid environment at high temperature (see Fig. 7).

We have shown in a separate article that Ti metal heat-treated after H_2SO_4/HCl mixed acid treatment without being subjected to NaOH treatment formed TiO_2 layer [20] that formed apatite on its surface in a body environment and tightly bonded to living bone. The formation of apatite on the surface was also attributed to positive surface charge [20]. Our present results show that apatite formation induced by the positive surface charge of the Ti metal heat-treated after acid treatment is not specific to the use of a mixed acid solution, but is independent of the type of acid solution used, and is also independent of the surface condition before the acid treatment.

It has been shown previously that a porous Ti metal heat-treated after an HCl treatment following an NaOH treatment exhibits high osteoconductivity [22, 42] as well as osteoinductivity [23, 24]. This type of bioactive porous Ti metal is now being subjected to clinical trials for application in spinal fusion devices after animal experiment [25]. Our results provide a contribution to the development of porous Ti metals with higher osteoconductivity and osteoinductivity.

5 Conclusions

Nanometer-scale roughness was generated on the surface of Ti metal by NaOH treatment and remained even after formation of titanium oxide by the subsequent acid and heat treatments, as long as the acid concentration was not high. Ti metal treated with HCl, HNO_3 and H_2SO_4 solutions after an NaOH treatment showed high apatite-forming ability in an SBF after a subsequent heat treatment, especially when the acid concentration was greater than 10 mM. This was not attributed to either the surface roughness induced by the chemical treatment or to the specific crystalline phase precipitated on the Ti metal, but to the positive surface charge of the TiO_2 precipitated on the Ti metal by the acid and heat treatments. The positively charged Ti metals first adsorbed the negatively charged phosphate ions in an SBF, and then the positively charged calcium ions to form apatite on their surface.

Such treated Ti metals are expected to form apatite on their surface in a short period, even in a living body, and can bond to living bone through this apatite layer. These findings will contribute to the development of porous Ti metal with higher osteoconductivity and osteoinductivity.

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