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Effect of HCl concentrations on apatite-forming ability of NaOH–HCl- and heat-treated titanium metal

**Deepak K. Pattanayak · Takahiro Kawai ·
Tomiharu Matsushita · Hiroaki Takadama ·
Takashi Nakamura · Tadashi Kokubo**

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Abstract Titanium (Ti) metal was treated with water or HCl solutions after 5 M NaOH solution treatment and then subjected to heat treatment at 600°C. The apatite-forming abilities of the treated Ti metals were examined in simulated body fluid. The apatite-forming ability of the Ti metal subjected to NaOH, water and heat treatment was lower than that of just NaOH and heat treatments. Ti metals subjected to NaOH, HCl and heat treatment showed apatite-forming abilities, which increased with increasing HCl concentrations up to the same level as that of NaOH- and heat-treated Ti metal. The former did not show a decrease in its apatite-forming ability, even in a humid environment for a long period, whereas the latter decreased its ability. The increase in the apatite-forming ability with increasing HCl concentrations suggests a different mechanism of apatite formation from that previously proposed.

D. K. Pattanayak (✉) · T. Matsushita · H. Takadama ·
T. Kokubo
Department of Biomedical Sciences, College of Life and Health Sciences, Chubu University, 1200 Matsumoto-cho, Kasugai, Aichi 487-8501, Japan
e-mail: deepak@isc.chubu.ac.jp;
deepak_pattanayak@rediffmail.com

T. Kawai
Department of Chemistry and Chemical Engineering, Graduate School of Science and Engineering, Yamagata University, 4-3-16 Jonan, Yonezawa, Yamagata 992-8510, Japan

T. Nakamura
Department of Orthopaedic Surgery, Graduate School of Medicine, Kyoto University, Kawahara-cho 54, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

1 Introduction

It has been shown that titanium (Ti) metal subjected to NaOH and heat treatments to form a sodium titanate on its surface spontaneously bonds to living bone through an apatite layer formed on its surface in the living body [1, 2]. This bioactive Ti metal was applied to an artificial hip joint, which has been in clinical use in Japan since 2007.

Ti metal subjected to NaOH, water and heat treatments to form Na-free titanium oxide on its surface was also found to bond to living bone through an apatite layer formed on its surface in the living body [3, 4]. Porous Ti metal subjected to these treatments showed both osteoconductivity and osteoinductivity [5]. The osteoinductivity of the porous Ti metal was increased by replacing the water with 0.5 mM HCl solution [6]. A spinal implant of the prepared osteoinductive porous Ti metal is now being subjected to clinical trials [7].

Based on these findings, it is expected that the osteoinductivity of the porous Ti metal can be further increased by using HCl solutions of higher concentrations. In the present study, the effect of differing HCl concentrations on the apatite-forming ability in a simulated body fluid (SBF) of Ti metal subjected to NaOH, HCl and heat treatments was investigated. It is reported that the osteoinductivity of porous materials is related to apatite formation on its surface in the living body [8], and that apatite formation on a material in the living body is reproduced, even in SBF [9].

For the practical applications of using a material as a bone substitute, it is required to exhibit a high apatite-forming ability, not only immediately after preparation, but after storage in a humid environment for long periods, and also to show high scratch resistance against hard surgical devices. In the present study, these properties were examined for the Ti metals subjected to the NaOH, HCl and heat treatments.

Concerning the apatite formation on Na-free titanium oxides, several papers have looked at titanium oxides prepared by different methods [10–22]. One of them proposed that apatite formation was epitaxially induced on a lattice plane of the rutile or anatase phase [21]. There is, however, no experimental evidence for this. Based on the present study of the effects of HCl concentration on the apatite-forming ability of NaOH-, HCl- and heat-treated Ti metals, a different mechanism of apatite formation on titanium oxide is proposed.

2 Materials and methods

2.1 Preparation of the specimens

Commercially pure Ti metal (Kobe Steel, Ltd., Kobe, Japan) was cut into rectangular specimens 10 × 10 × 1 mm^3 in size and polished with #400 diamond plates. All polished specimens were 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. They were soaked in 5 ml of 5 M NaOH solution at 60°C for 24 h in an oil bath shaken at 120 strokes/min, and gently washed with ultra-pure water. Some of them were soaked in 400 ml of ultra-pure water or 10 ml of HCl solutions at concentrations of 0.5, 50 or 100 mM. All of these specimens were heated at 40°C for 24 h in an oil bath shaken at 120 strokes/min. The specimens were gently washed with ultra-pure water and dried overnight in an oven at 40°C. Some of the specimens were heated up to 600°C at a rate of 5°C/min in a Fe–Cr–Al electric furnace under an air atmosphere, maintained at this temperature for 1 h and then naturally cooled to room temperature in the furnace.

2.2 Examination of the structures and properties of surfaces

The surfaces and cross-sections of the Ti metals subjected to various treatments described above were coated with Pt/Pd and observed under field emission scanning electron microscope (FE-SEM; Hitachi S-4300, Japan).

The chemical compositions of the surfaces of the treated Ti metals were analyzed by an energy dispersive X-ray analyzer (EMAX; Horiba Ltd., Japan) attached to the FE-SEM under an acceleration voltage of 9 kV. This analysis was performed at five different areas and their average value calculated.

The crystalline phases, which formed on the surfaces of the treated Ti metals, were examined by thin film X-ray diffraction (TF-XRD; RINT-2500, Rigaku Co., Japan). In this measurement, the X-ray source was Cu Kα, which was emitted at a current density of 200 mA and an accelerating voltage of 50 kV. The angle of the incident beam was 1° against the specimen surface.

In order to identify the crystalline phases, laser Raman spectroscopy was also performed on the surfaces of the treated Ti metals (FT-Raman; LabRAM HR-800, Horiba Jobin Yvon, France). For this Raman spectroscopy, an argon (Ar) laser with a wavelength of 514.5 nm was used.

The scratch resistances of the surfaces of the treated Ti metals were measured using a scratch tester (CSR-2000, Rhesca Co., Ltd., Japan). In this measurement, a stylus with diamond tip moved on the sample surface at a speed of 10 mm/s and an applied load of 100 mN/min. The critical scratch load was calculated from the abrupt change in the signal of the sensor output, which indicates a complete peeling of the surface layer.

2.3 Examination of the apatite-forming ability in SBF

The treated Ti metals were soaked in 30 ml of an acellular SBF 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, SO_4^{2-} 0.5 mM) nearly equal to those of human blood plasma at 36.5°C [9]. 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) into ultra-pure water, and buffered at pH 7.4 with tris-hydroxymethylaminomethane ($(CH_2OH)_3CNH_2$) and 1 M HCl (Nacalai Tesque, Inc.). After 1 and 3 days soaking, the specimens were removed from SBF, gently washed with ultra-pure water and dried in an oven at 40°C. Apatite formation on their surfaces was examined by TF-XRD and FE-SEM.

In order to examine the stability of the apatite-forming ability in a humid environment, the treated Ti metals were kept in 95% relative humidity at 80°C for 2 weeks, and the apatite formation on their surfaces in SBF was examined by TF-XRD and FE-SEM.

3 Results

3.1 The surface structures of treated Ti metals

Figure 1 shows FE-SEM photographs of the surfaces and cross-sections of Ti metals subjected to NaOH (A), NaOH–water (A-W), NaOH–0.5 mM HCl (A-05HC), NaOH–50 mM HCl (A-50HC), and NaOH–100 mM HCl (A-100HC) treatments. Figure 2 shows the same specimens subsequently subjected to the heat treatments. It can be seen from Fig. 1 that a network structure consisting of fine lath-like phases, 1 μm in length and elongated perpendicular

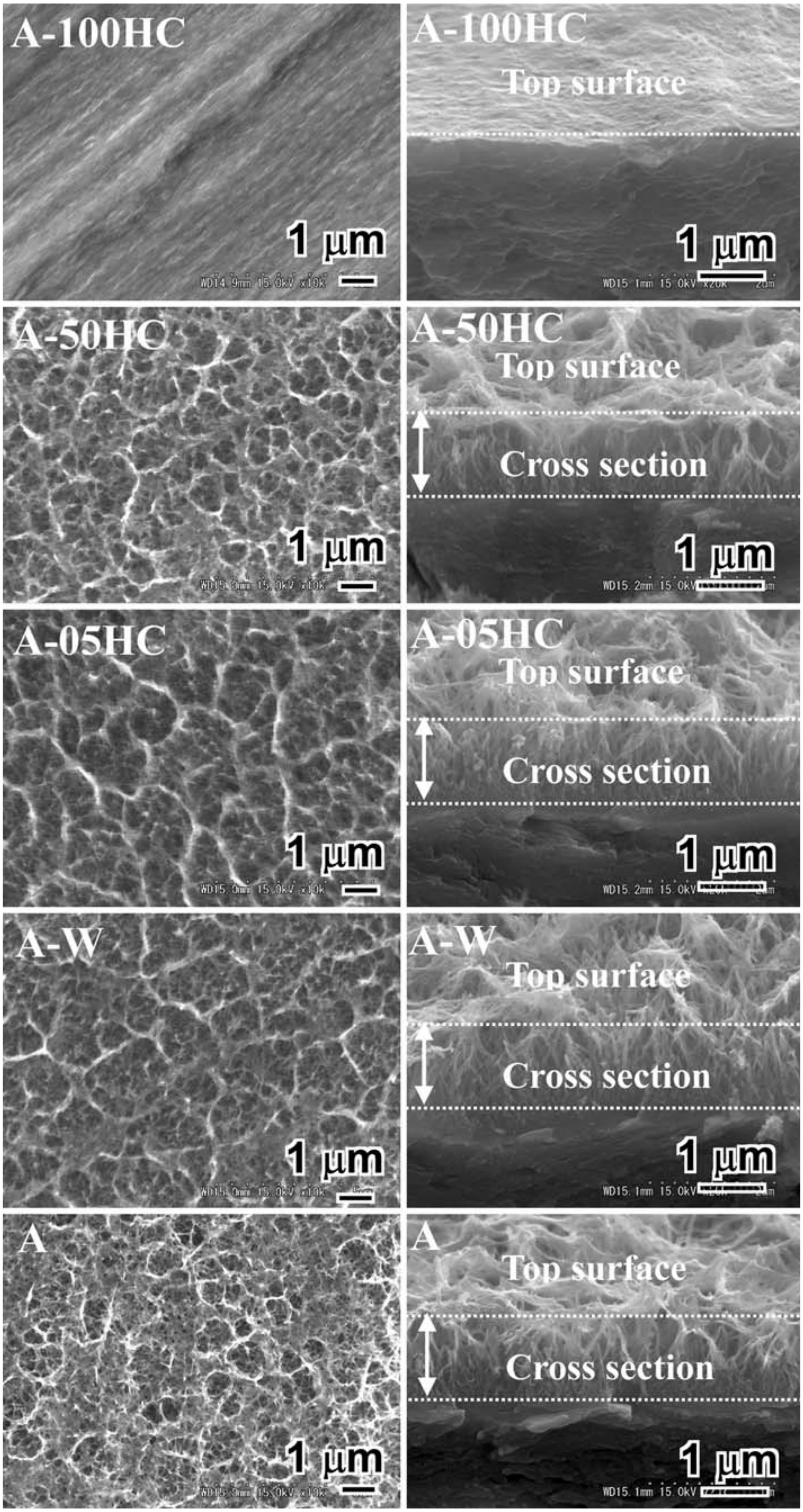


Fig. 1 FE-SEM photographs of surfaces (*left hand side*) and cross-sections (*right hand side*) of Ti metals which were subjected to NaOH (A), NaOH–water (A-W), NaOH–0.5 mM HCl (A-05HC), NaOH–50 mM HCl (A-50HC), or NaOH–100 mM HCl (A-100HC) treatments

to the surface was formed on the surfaces of the Ti metals by the first NaOH treatment. This network surface structure was not essentially changed by subsequent treatments with water or HCl solutions less than 50 mM in concentration, and was completely dissolved by the treatment with the 100 mM HCl solution. All these surface structures were not essentially changed by the final heat treatment, as seen in Fig. 2.

Energy dispersive X-ray analysis of the surfaces of the treated Ti metals showed that about 5.5 at.% of Na was incorporated into the surface of the Ti metal by the first NaOH treatment, and then completely removed by subsequent water or HCl treatments.

Figures 3 and 4 show TF-XRD patterns and Raman spectra of the surfaces of the Ti metal treated with the NaOH, water or HCl solutions with different concentrations. It can be seen that sodium hydrogen titanate ($Na_xH_{2-x}Ti_3O_7$; $0 < x < 2$) [23] was precipitated on the surface of the Ti metal by the first NaOH treatment. It was transformed into hydrogen titanates ($H_2Ti_3O_7$) [24] by the subsequent treatments with water or HCl solutions less than 50 mM in concentration, but completely dissolved by the treatment with the 100 mM HCl solution.

Figures 5 and 6 show TF-XRD patterns and Raman spectra of the surfaces of the same specimens subsequently subjected to the heat treatment. It can be seen from these figures that the sodium hydrogen titanate precipitated on the Ti metal by the first NaOH treatment was transformed into sodium titanate ($Na_2Ti_6O_{13}$) [23, 24] and rutile by the subsequent heat treatment. The hydrogen titanate precipitated on the Ti metal by NaOH and water or HCl treatments was transformed into anatase and rutile by the subsequent heat treatment. The amount ratio of rutile to anatase increased from 0.5 to 50 mM with increasing concentrations of the HCl solutions. The Ti metal subjected to NaOH and 100 mM HCl solution only formed rutile on its surface after the subsequent heat treatment.

3.2 Scratch resistance of the surfaces of treated Ti metals

Figure 7 shows the critical scratch resistance of the surfaces of Ti metals subjected to NaOH and water or HCl treatments, as well as those of the same specimens subsequently subjected to heat treatment. It can be seen from Fig. 7 that the scratch resistances of Ti metal surfaces are as low as about 5 mN before heat treatment, but markedly increase up to 45 to 55 mN by the subsequent heat treatment, irrespective of the kind of chemical treatments.

3.3 Apatite-forming ability of the treated Ti metals in SBF

Figure 8 shows FE-SEM photographs of the surfaces of Ti metals soaked in SBF for 1 and 3 days after NaOH and water or HCl treatments. It can be seen from Fig. 8 that a low apatite-forming ability is induced by the first NaOH treatment. This ability is lost by the subsequent water or 0.5 mM HCl treatment, but a little is recovered by HCl treatments of 50 mM and higher concentrations.

Figure 9 shows FE-SEM photographs of the surfaces of Ti metals soaked in SBF for 1 and 3 days after the chemical treatments and subsequent heat treatment. It can be seen from Fig. 9 that the apatite-forming ability of all the chemically treated Ti metals was increased by the heat treatment. Among the heat-treated Ti metals, those that were only subjected to NaOH treatment and those subjected to NaOH and HCl treatments 50 mM and higher concentration gave remarkably high apatite-forming abilities. However, those subjected to NaOH and water or HCl treatments lower than 0.5 mM in concentration gave relatively low apatite-forming abilities.

Figure 10 shows FE-SEM photographs of the surfaces of Ti metals subjected to NaOH and heat treatments or NaOH, 50 mM HCl and heat treatments, and which were kept in a relative humidity of 95% at 80°C for 2 weeks and soaked in SBF for 1 day. It can be seen from Fig. 10 that the latter Ti metal shows a high apatite-forming ability, even after being kept in high humid environment for 2 weeks, whereas the former Ti metal drastically decreased its apatite-forming ability after being kept in the same environment.

4 Discussion

It has been reported that materials able to form apatite on their surfaces in SBF form apatite in the living body, and bond to living bone through this apatite layer [9]. It is apparent from Fig. 8 that this apatite-forming ability is induced on the Ti metal by NaOH treatment. However, its apatite-forming ability is not high and its scratch resistance is very low (Fig. 7).

When it was heat treated at 600°C, the resultant product showed a remarkably higher apatite-forming ability (Fig. 9), as well as a higher scratch resistance (Fig. 7). Therefore, these treatments were applied to a porous Ti metal layer of an artificial hip joint, and the resultant joint has been clinically used in Japan since 2007. However, the apatite-forming ability of the NaOH- and heat-treated Ti metal is not stable under high humid environments for long periods (Fig. 10). Some modification of the chemical treatment was required for the clinical application.

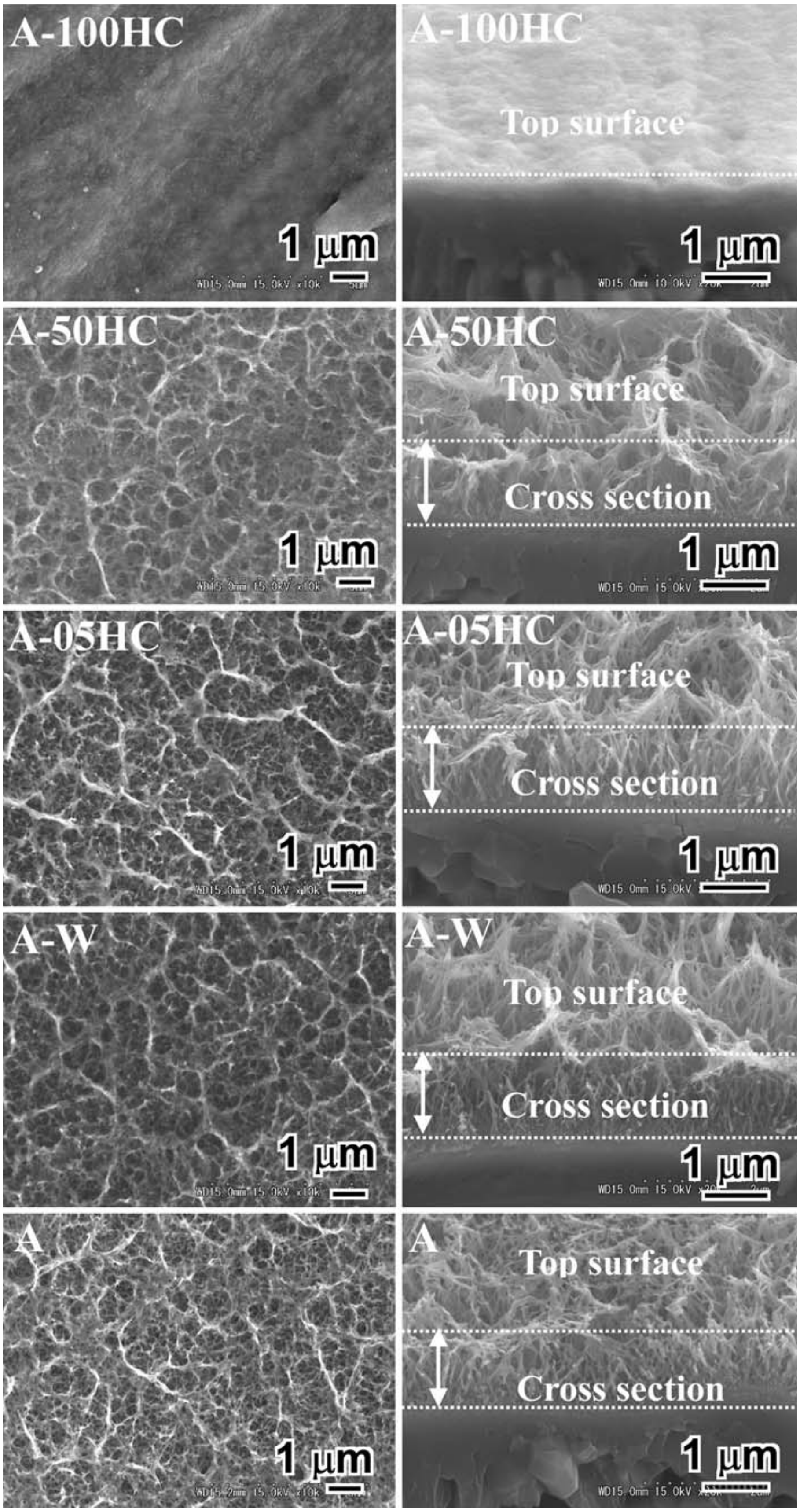


Fig. 2 FE-SEM photographs of surfaces (*left hand side*) and cross-sections (*right hand side*) of Ti metals which were subjected to NaOH (A), NaOH–water (A-W), NaOH–0.5 mM HCl (A-05HC), NaOH–50 mM HCl (A-50HC), or NaOH–100 mM HCl (A-100HC) treatments, and then subjected to heat treatment

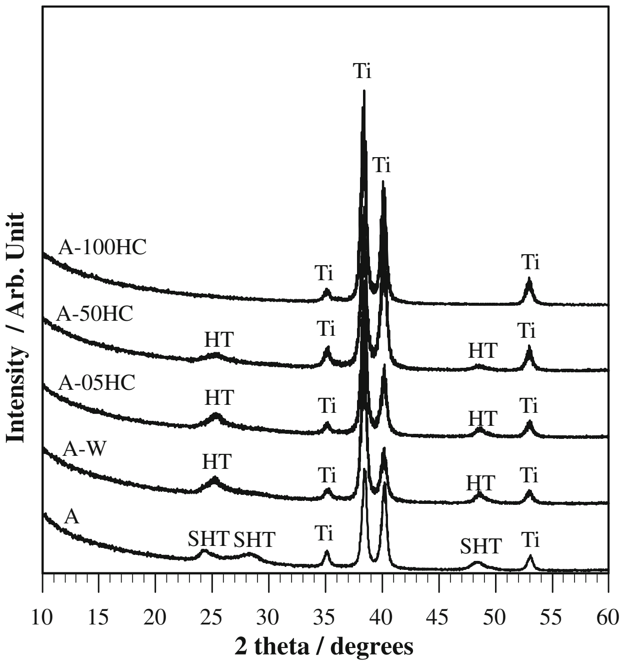


Fig. 3 TF-XRD patterns of surfaces of Ti metals which were subjected to NaOH (A), NaOH–water (A-W), NaOH–0.5 mM HCl (A-05HC), NaOH–50 mM HCl (A-50HC), or NaOH–100 mM HCl (A-100HC) treatments. *Ti* α Ti, *HT* hydrogen titanate, *SHT* sodium hydrogen titanate

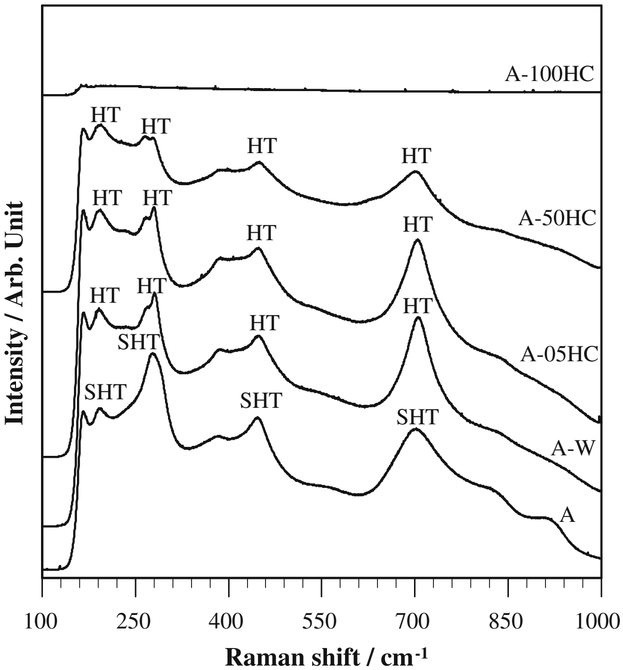


Fig. 4 Raman spectra of surfaces of Ti metals which were subjected to NaOH (A), NaOH–water (A-W), NaOH–0.5 mM HCl (A-05HC), NaOH–50 mM HCl (A-50HC), or NaOH–100 mM HCl (A-100HC) treatments. *HT* hydrogen titanate, *SHT* sodium hydrogen titanate

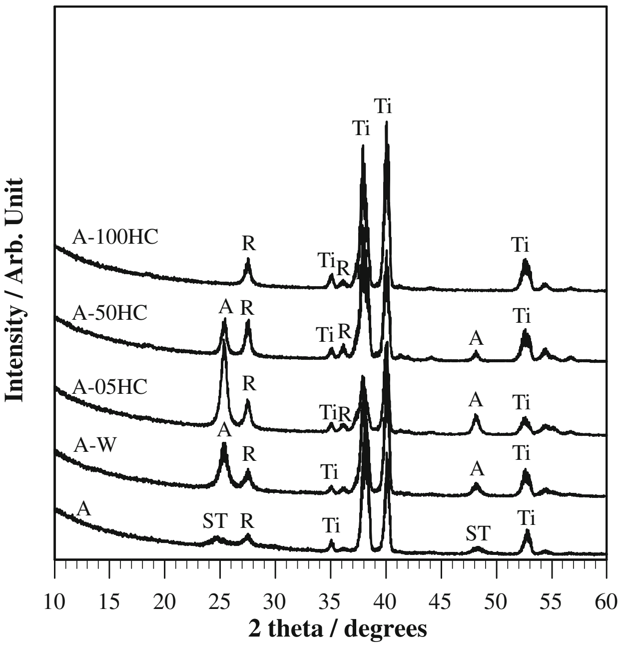


Fig. 5 TF-XRD patterns of surfaces of Ti metals which were subjected to NaOH (A), NaOH–water (A-W), NaOH–0.5 mM HCl (A-05HC), NaOH–50 mM HCl (A-50HC), or NaOH–100 mM HCl (A-100HC) treatments, and then subjected to heat treatment. *Ti* α Ti, *ST* sodium titanate, *A* anatase, *R* rutile

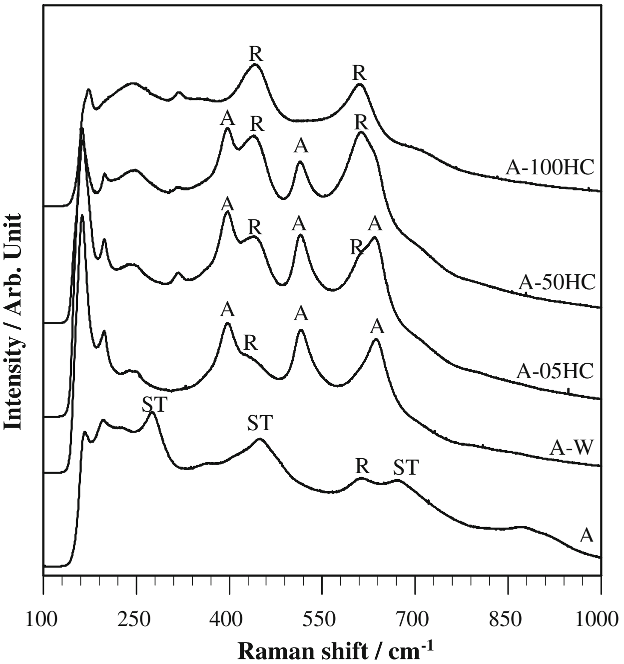
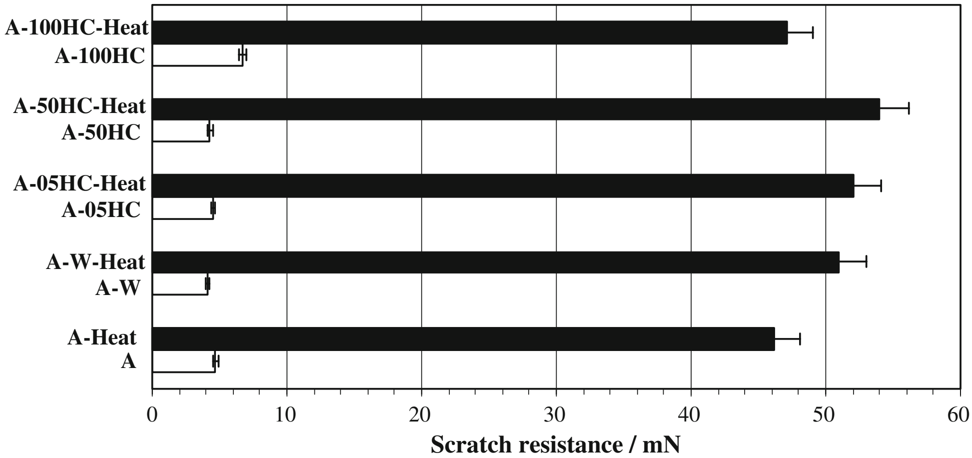


Fig. 6 Raman spectra of surfaces of Ti metals which were subjected to NaOH (A), NaOH–water (A-W), NaOH–0.5 mM HCl (A-05HC), NaOH–50 mM HCl (A-50HC), or NaOH–100 mM HCl (A-100HC) treatments, and then subjected to heat treatment. *ST* sodium titanate, *A* anatase, *R* rutile

Fig. 7 Scratch resistances of surfaces of Ti metals which were subjected to NaOH (A), NaOH–water (A-W), NaOH–0.5 mM HCl (A-05HC), NaOH–50 mM HCl (A-50HC), or NaOH–100 mM HCl (A-100HC) treatments, and those subjected to the subsequent heat treatment



It is apparent from Figs. 7, 9 and 10 that Ti metal subjected to NaOH, 50 mM HCl and heat treatments exhibits not only a remarkably high apatite-forming ability and high scratch resistance, but also high long-term stability of its apatite-forming ability, even in a humid environment. Thus, prepared Ti metal is believed to be useful as bone substitute. According to Fig. 9, Ti metal subjected to NaOH and 100 mM HCl treatment also gives a high apatite-forming ability after the subsequent heat treatment. However, the adhesive strength of the apatite layer formed on the Ti metal in SBF was poor. This is because the Ti metal lacks a fine network structure on its surface (Fig. 2), and hence, treated Ti metal is not useful as bone substitute.

Osteoinductivity was reported to be higher for porous Ti metals subjected to NaOH, water and heat treatments than for those subjected to NaOH and heat treatments, contrary to their apatite-forming abilities (Fig. 9). The lower osteoinductivity of the NaOH- and heat-treated Ti metal might be attributed to the adverse effect of Na^+ ions released from it on cell activities [5]. Osteoinduction of the NaOH-, 0.5 mM HCl- and heat-treated porous Ti metal was higher than that of the NaOH-, water- and heat-treated Ti metal [6]. This might be attributed to the higher apatite-forming ability of the former compared with the latter (Fig. 9). In view of this, porous Ti metals subjected to NaOH, 50 mM HCl and heat treatments are expected to show higher osteoinductivity, because they exhibit a higher apatite-forming ability.

The reasons why the NaOH- and heat-treated Ti metal shows a high apatite-forming ability is interpreted in terms of the electrostatic interaction of sodium titanate on its surface with the ions in the SBF [25] The sodium titanate releases Na^+ ions by exchange with H_3O^+ ions in the SBF to form Ti–OH groups on its surface, locally increasing the pH of the surrounding fluid. In such a high pH environment, the Ti–OH groups are negatively charged, as shown in Fig. 11 [26, 27], to react with positively charged Ca^{2+} ions. As the Ca^{2+} ions accumulate on its surface, the surface is positively charged to react with the negatively charged phosphate ions to form a calcium phosphate. Thus, the formed calcium phosphates take an amorphous structure when formed, but then transform into more stable crystalline apatite. According to the present results, Ti metals subjected to NaOH, 50 mM HCl and heat treatments show the same level of high apatite-forming ability as that of Ti metals subjected to NaOH and heat treatments, in spite of a lack of Na^+ ions on its surface. How could this be interpreted?

All the Ti metals treated with NaOH, water or HCl solutions precipitated titanium oxide on their surfaces after the subsequent heat treatment (Figs. 5 and 6). However, they did not show the same level of apatite-forming abilities. The NaOH, water-treated Ti metal showed the lowest apatite-forming ability. The NaOH, HCl-treated Ti metal shows higher apatite-forming abilities, which increase with increasing HCl concentrations (Fig. 9). A fine network structure was observed on the surfaces of the Ti metals heat treated after treatment with water or HCl solutions at concentrations of 50 mM or less, but not on the surface of the Ti metal heat treated after the treatment with 100 mM HCl solution (Fig. 2). This network structure is, therefore, not responsible for the apatite formation on the surface of the Ti metal.

The ratio of rutile to anatase on the surface of heat-treated Ti metals increased with increasing HCl concentrations, as shown in Fig. 5. Their apatite-forming abilities were, however, not proportional to the rutile/anatase ratio. Ti metal treated with 50 mM HCl solution gave almost the same high apatite-forming ability as that of Ti metal treated with 100 mM HCl solution (Fig. 9), although the former precipitated both the rutile and anatase, and the latter only precipitated rutile (Fig. 5). Therefore, apatite formation on Ti metal is not governed by the type of titanium oxide on its surface.

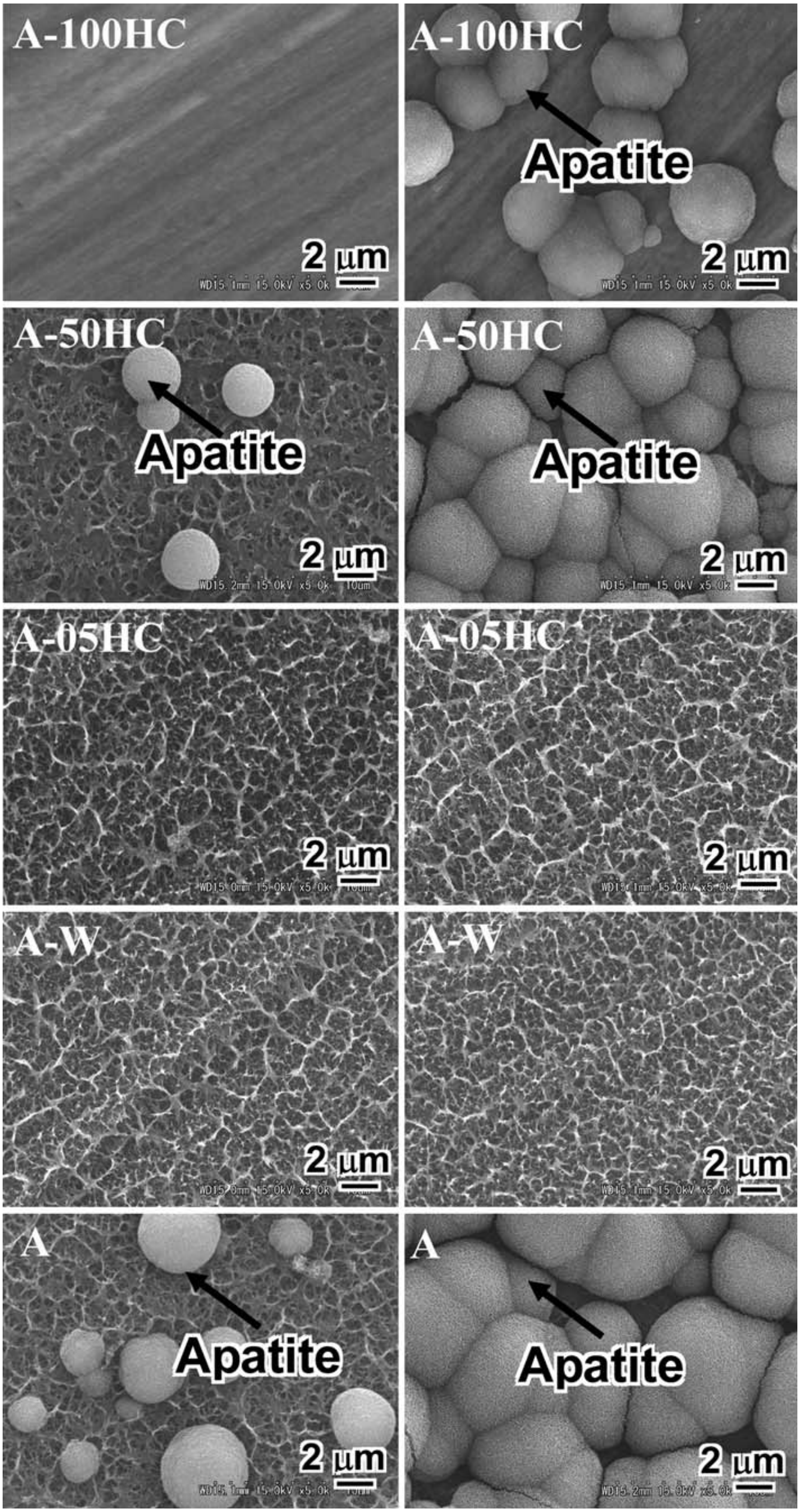


Fig. 8 FE-SEM photographs of surfaces of Ti metals which were subjected to NaOH (A), NaOH–water (A-W), NaOH–0.5 mM HCl (A-05HC), NaOH–50 mM HCl (A-50HC), or NaOH–100 mM HCl (A-100HC) treatments, and subsequently soaked in SBF for 1 (*left hand column*) and 3 days (*right hand column*)

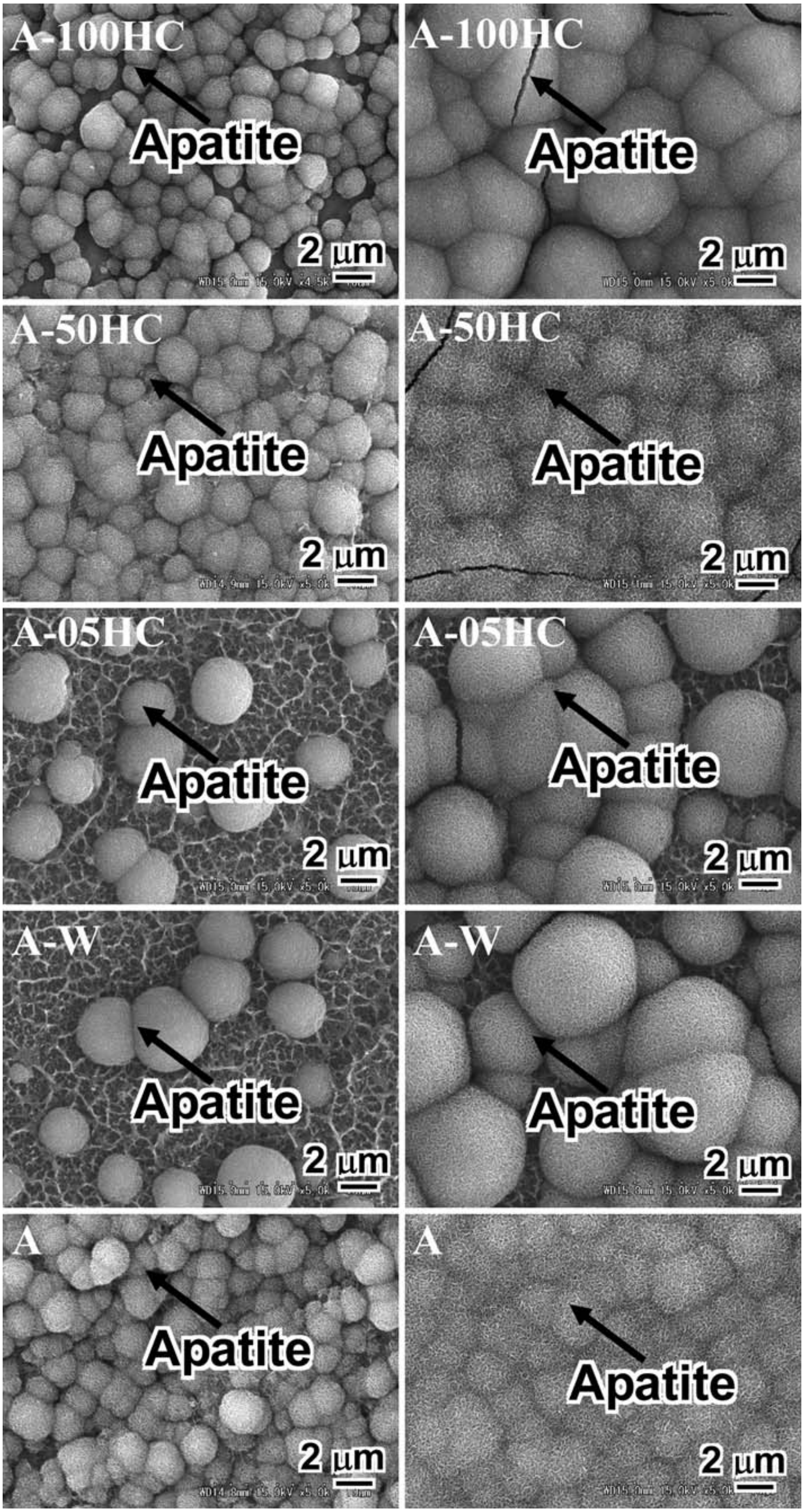


Fig. 9 FE-SEM photographs of surfaces of Ti metals which were subjected to heat treatment after NaOH (A), NaOH–water (A-W), NaOH–0.5 mM HCl (A-05HC), NaOH–50 mM HCl (A-50HC), or NaOH–100 mM HCl (A-100HC) treatments, and subsequently soaked in SBF for 1 (*left hand column*) and 3 days (*right hand column*)

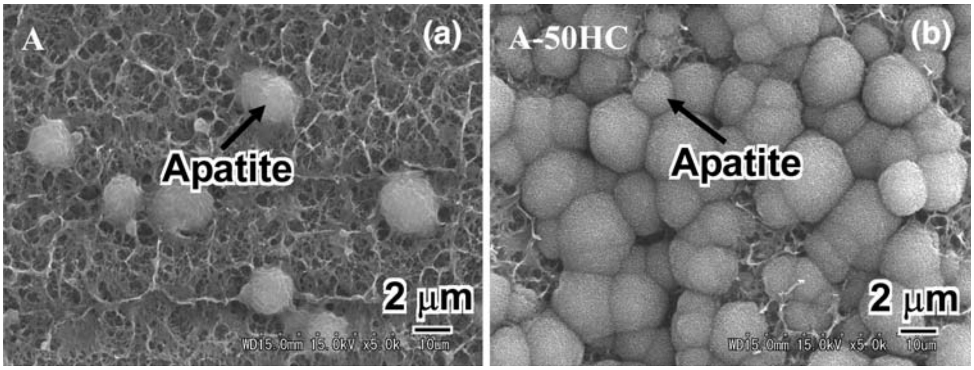


Fig. 10 FE-SEM photographs of surfaces of Ti metals subjected to NaOH and heat treatments (**a**), or NaOH–50 mM HCl and heat treatments (**b**), both of which were soaked in SBF for 1 day, after kept in 95% humidity at 80°C for 2 weeks

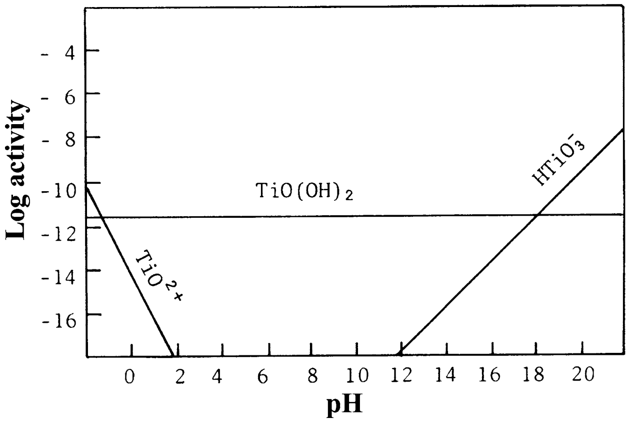


Fig. 11 Stability of titanium oxide in aqueous solution at different pHs (25°C)

Different mechanisms of apatite formation on titanium oxide must be considered. A possible mechanism for the apatite formation is electrostatic interaction of the titanium oxide with the ions in the SBF. According to Fig. 11, titanium oxide is positively charged in the acidic region and its positive charge increases with decreasing pH. If titanium oxide is positively charged, it could combine with the negatively charged phosphate ions in SBF. As the phosphate ions accumulate, its surface is negatively charged to combine with the positively charged Ca^{2+} ions to form calcium phosphates. Thus, the formed calcium phosphate might eventually be transformed into apatite. The Ti metal treated with HCl solutions after the NaOH treatment gave hydrogen titanate or Ti metal, and then transformed them into anatase and/or rutile of the titanium oxide by the subsequent heat treatment. If a certain kind of surface structure specific to the acid solutions is formed on the Ti metal by the HCl treatments, and retained even after the subsequent heat treatment, titanium oxide formed by the subsequent heat treatment could exhibit a positive surface charge in an aqueous solution.

HCl solutions 0.5, 50 and 100 mM in concentration give pH levels of 3.45, 1.49 and 1.16, respectively. The titanium oxide formed on Ti metals treated with 0.5, 50 and 100 mM HCl solutions by the subsequent heat treatment could give positive surface charges, which increase with increasing HCl concentrations. As a result, the apatite-forming ability of the titanium oxide increases with increasing HCl concentration. This assumption will be discussed in more detail in a separate paper and will be based on experimental data, such as the Zeta potential and XPS spectra of the surfaces of the treated Ti metals.

5 Summary

Ti metal was treated with water or HCl solutions after 5 M NaOH treatment, and then subjected to heat treatment at 600°C. Sodium hydrogen titanate, which was precipitated on the surface of Ti metal by the NaOH treatment, transformed into hydrogen titanate by the treatment with water or 0.5 mM and 50 mM HCl solutions, and dissolved by the treatment with 100 mM HCl solution. All the chemically treated Ti metals precipitated anatase and/or rutile on their surfaces after heat treatment.

The scratch resistances of the surfaces of the chemically treated Ti metals were low before the heat treatment, and were significantly improved by the heat treatment.

The apatite-forming abilities in SBF of the Ti metals were low before the heat treatment, but were remarkably increased by the heat treatment. The apatite-forming ability of the Ti metal subjected to NaOH, water and heat treatment was much lower than that of the Ti metal subjected to the NaOH and heat treatments. Ti metals treated with NaOH, HCl solutions and heat treatment, however, showed apatite-forming abilities, which increased with increasing HCl concentrations up to the same level as that of the NaOH- and heat-treated Ti metal. The NaOH–HCl- and heat-treated Ti metal did not show a decrease in apatite-forming ability, even under a humid environment for a long period, although the NaOH- and heat-treated Ti metal showed the decrease.

The apatite-forming abilities of the former, which increased with increasing HCl concentrations, might be interpreted in terms of the electrostatic reaction of the positively charged titanium oxide on their surfaces with the ions in the SBF.

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