Surface Modification of Titanium with Hydrothermal Treatment at High Pressure

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Surface modification of titanium was investigated by means of hydrothermal treatment with a maximum pressure of 6.3 MPa (280°C temperature) in CaO solution or water to improve bioactivity and biocompatibility. As a result, calcium titanate was formed on the titanium surface. Moreover, titanium oxide and titanium hydroxide layers on the surface increased as temperature and pressure increased. The surface-modified titanium was also immersed in a simulated body fluid (SBF) to estimate its bioactivity. Needle-like apatite precipitation was observed on all hydrothermal-treated titanium surfaces after immersion in SBF for four weeks. In particular, the apatite precipitation of titanium treated with 6.3 MPa in CaO solution was clearer and larger in amount than those of all other hydrothermal-treated specimens. Further, the amount of precipitate corresponded to the thickness of the surface-modified layer and the amount of calcium in the surface layer. The results suggested that surface modification of titanium with high-pressure hydrothermal treatment seemed to improve bioactivity and biocompatibility.

Key words: Titanium, Surface modification, Hydrothermal treatment

INTRODUCTION

Titanium and its alloys have become popular as biomaterials in the dental and medical fields because of their excellent mechanical properties and biocompatibility^{1–13}. In particular, titanium materials for implantation of artificial tooth roots have been widely employed because of their osteointegration with bone tissues. However, titanium materials are less able to facilitate osteointegration or osteobonding than calcium phosphate materials such as apatites, calcium phosphate cements, bioglasses, and bioglass-ceramics. Therefore, various surface modification methods of titanium have been investigated and introduced in a bid to improve the activity of osteointegration^{1,14–18}. These methods can be roughly classified into two types – namely, chemical and physical processes. A common physical method for the surface modification of titanium substrates is plasma-spraying of calcium phosphate particles such as hydroxyapatite or tricalcium phosphate. Moreover, calcium ion implantation and apatite blast coating have been investigated as alternative physical methods for modifying titanium^{19–22}. On the other hand, chemical surface modification of titanium substrates by means of treatment in solutions such as NaOH, H₂O₂, Ca(OH)₂ have also been investigated^{1,23–27}.

When bioactive materials such as hydroxyapatite

are implanted into bone, they bond to living bone in the body via a bone-like apatite layer formed on their surface^{4,16,27,28} – which is immediately precipitated from body fluid, forming a chemical bond with the bone. *In vivo* and *in vitro* investigations have revealed that the bone-like apatite precipitation on pure titanium is delayed in comparison with calcium phosphate materials. However, apatite precipitation on surface-modified titanium is more rapid than on pure titanium^{14,15}. We previously investigated surface modification of titanium by a simple method using immersion in calcium-containing solutions¹⁴. In that study, the surface-modified titanium effectively precipitated apatite *in vitro*, thus illustrating that surface-modified titanium is possibly superior to unmodified titanium in terms of bone conduction. Moreover, the titanium surface-modified by immersion in calcium-containing solutions was investigated by using an autoclave for sterilization (121°C, 0.2 MPa)¹¹. This was because in comparison to a simple immersion method, modification of titanium in a calcium-containing solution with hydrothermal treatment at low temperature and pressure (121°C, 0.2 MPa) was expected to result in better osteointegration. Therefore, we considered that hydrothermal treatments (in calcium-containing solution) using an autoclave with a maximum pressure 6.3 MPa and temperature of 280°C might improve bioactivity such as the osteointegration of titanium.

Further, the results of our previous investigation¹⁴⁾ led to the hypothesis that with increase of the titanium oxide or calcium titanate layer on the titanium surface, the osteointegration of titanium would improve accordingly.

In this study, surface modification of titanium was performed in calcium oxide (CaO) and distilled water with high-pressure hydrothermal treatment in a special autoclave. The surface structure of titanium surface-modified by hydrothermal treatments was then investigated by X-ray photoelectron spectroscopy, X-ray diffractometry, and scanning electron microscopy, and the modificatory effects of various hydrothermal treatments on the titanium surface thereby discussed. Moreover, the influence of hydrothermal treatment on the bioactivity of surface-modified titanium was examined by apatite precipitation with immersion in a simulated body fluid.

MATERIALS AND METHODS

Preparation of titanium specimens

Commercially pure titanium (99.5%) plates were used. Each plate was 1 mm in thickness and 33 mm in diameter. The plates were metallographically polished with SiC papers, and final polishing was performed with #800 SiC paper. After polishing, the

plates were ultrasonically washed in acetone, ethyl alcohol, and distilled water for 15 minutes each and dried in a desiccator under reduced pressure for five hours. Next, they were weighed with a microbalance (accuracy 0.01 mg) and their dimensions measured.

Surface modification

Experimental procedures used for surface modification in this research are shown in Fig.1. Hydrothermal treatment was performed using an autoclave (TVS-N2, Taiatsu Techno Co., Tokyo, Japan). The plates were treated by temperature and pressure conditions of 200°C and 1.4 MPa, or 280°C and 6.3 MPa, respectively, in the autoclave. These hydrothermal conditions were maintained for one hour. The liquid employed in the autoclave consisted of distilled water and 0.02 mol/L CaO aqueous solution. CaO is immediately transformed to calcium hydroxide, $\text{Ca}(\text{OH})_2$, in water. Therefore, CaO solution equals $\text{Ca}(\text{OH})_2$ solution. After treatment, the plates were rinsed in sterile distilled water, dried in a vacuum desiccator, and weighed again.

Immersion in SBF

For the simulated body fluid (SBF), Hanks' solution was prepared without organic species and the pH of the solution was adjusted to 7.4 with 7.5% NaHCO_3 .

Fig. 1 Surface-modification method and analyses in this study.

solution immediately after preparation. The final inorganic ion concentrations (mol/L) of the solution were 1.42×10^{-1} Na^+ , 5.81×10^{-3} K^{2+} , 8.11×10^{-4} Mg^{2+} , 1.26×10^{-3} Ca^{2+} , 1.45×10^{-1} Cl^- , 7.78×10^{-4} HPO_4^{2-} , 8.11×10^{-4} SO_4^{2-} , and 4.17×10^{-3} CO_3^{2-} . Surface-modified titanium plates were immersed in 37°C SBF in a Teflon-sealed bottle for one week and four weeks. Volume of the solution was 125 ml per bottle and four plates were immersed in each bottle. Three plates were set vertically and one plate was laid at the bottom of the bottle. The SBF in the bottle was changed every three days to maintain the ionic concentrations because of precipitation from the SBF. No precipitates were confirmed to form in the solution or on the wall of the bottle. After immersion, the plates were rinsed in distilled water, dried in the vacuum desiccator, and weighed again. Mass change per unit surface area before and after immersion was determined.

Analysis of specimens

Specimen surface was characterized using X-ray photoelectron spectroscopy (XPS; Quantum 2000, PHI Co., USA) with an Al K α line. Argon ion sputtering was carried out during XPS to examine the composition and structure of the surface layer. Sputtering time was one minute at 3 keV and sputtering speed was approximately 37 nm/min for SiO_2 . The O 1s spectrum in XPS spectra contained three peaks originating from O^{2-} , OH^- , and hydrate and/or water (Fig. 2)²⁹⁾. Composition at three oxygen sites was evaluated according to the methods of Asami *et al.*^{30,31)}.

Fig. 2 O 1s XPS spectra obtained from titanium after hydrothermal treatment. 'I' indicates oxygen (O), 'II' indicates hydroxyl group (OH), and 'III' indicates water (H_2O).

A scanning electron microscope (SEM; JSM-5600LV, JEOL, Tokyo, Japan) was used to observe the microstructure on the outermost surface layer of the specimens before and after immersion in SBF. For SEM observation, the specimens were sputtered with gold. The crystal phase of each specimen was analyzed using X-ray diffraction (XRD; Miniflex, Rigaku Co., Tokyo, Japan). The XRD conditions were Ni monochromatized $\text{CuK}\alpha$ radiation ($\lambda=0.1540$ nm) generated at 30 kV and 15 mA.

The influence of hydrothermal treatment on the mechanical properties of titanium specimens was also investigated in terms of tensile strength. Tensile strength of the specimens (1 mm ϕ $\times$ 50 mm) was measured using a universal testing machine (Auto-graph AGS-500A, Shimadzu Co., Kyoto, Japan) with a crosshead speed of 0.5 mm/min.

RESULTS

SEM micrograph of non-treated titanium specimen (as a control) is shown in Fig. 3, while those of hydrothermal-treated specimens at 200°C (1.4 MPa) and 280°C (6.3 MPa) in CaO solution and distilled water are shown in Fig. 4. No significant differences between before and after several hydrothermal treatments were confirmed by SEM observations of the surface structure on specimens. Fig. 5 shows the XRD patterns of titanium specimens before and after several hydrothermal treatments. Non-treated specimen had XRD peaks only for titanium (Ti) crystals and no peaks were confirmed for the other crystals. After the titanium specimens were treated at 200°C

Fig. 3 Scanning electron micrograph (SEM) showing the surface of non-treated titanium specimen.

Fig. 4 Scanning electron micrographs (SEM) showing the surface of titanium specimens with hydrothermal treatment (HT). A: HT at 200°C in water; B: HT at 280°C in water; C: HT at 200°C in CaO solution; and D: HT at 280°C in CaO solution.

(1.4 MPa) or 280°C (6.3 MPa) in CaO solution, small peaks for anatase (TiO_2) and calcium titanate (CaTiO_3) were fractionally detected by XRD. Moreover, a small XRD peak for anatase (TiO_2) was also confirmed for the specimen treated at 280°C (6.3 MPa) in distilled water.

Table 1 shows the compositions of surface layers on the hydrothermal-treated specimens, calculated from XPS spectra. In the specimen treated at 200°C in distilled water, the outermost layer had a composition of 33% titanium (Ti) and 66% oxygen (O), that is, almost pure TiO_2 . The oxygen component of the specimen treated at 280°C was greater than that at 200°C. After sputtering, composition of the second layer on the specimen surfaces treated in water were the same as those of the outermost layers. Surface layers of specimens treated in CaO solution were composed of calcium (Ca) and oxygen, and there was almost no titanium in the outermost and second surface layers. The relations of oxygen (O), hydroxyl group (OH), and water (H_2O) contents on the

surfaces of hydrothermal-treated specimens, calculated from O 1s XPS spectra, are shown in Table 2. In the outermost surface layer for all hydrothermal treatments, the $(\text{H}_2\text{O} + \text{OH})/\text{O}$ ratio of the titanium surfaces increased more than the OH/O ratio. The OH group content on the outermost surface layer increased with treatment in CaO solution as compared with treatment in water. The OH group with treatment in CaO solution might be due to adsorption of $\text{Ca}(\text{OH})_2$ because titanium constituent did not exist in the surface layer (Table 1). The influences of temperature and pressure of hydrothermal treatment on the OH group and H_2O contents could not be confirmed. After sputtering with argon, the OH group content in the second surface layer decreased more than that in the outermost surface layer. In particular, for specimens treated in CaO solution, their OH group content markedly decreased with sputtering.

Fig. 6 shows the SEM observations of titanium specimens with hydrothermal treatment (A, B, C, D) and without treatment (N) after immersion in SBF

Table 1 Surface composition of titanium specimens after hydrothermal treatment (XPS)

Outermost layer			Composition (atomic%)		
			Ti	Ca	O
200°C	1.4 MPa	Water	32.9	—	65.6
280°C	6.3 MPa	Water	20.1	—	78.4
200°C	1.4 MPa	CaO solution	—	12.7	87.4
280°C	6.3 MPa	CaO solution	4.1	33.9	62.1

After sputtering			Composition (atomic%)		
			Ti	Ca	O
200°C	1.4 MPa	Water	35.9	—	63.8
280°C	6.3 MPa	Water	20.4	—	79.0
200°C	1.4 MPa	CaO solution	—	19.3	80.7
280°C	6.3 MPa	CaO solution	2.0	16.6	81.3

Fig. 5 X-ray diffraction patterns of titanium specimens, before and after hydrothermal treatment (HT).

for one and four weeks. Almost no precipitates were observed on the non-treated specimens after immersion in SBF for one (N_1) and four weeks (N_4). With hydrothermal treatment, precipitates were confirmed on the surfaces of all specimens. However, there was little on the titanium treated in CaO solution at 200 °C after immersion for one week (C_1). All

hydrothermal-treated specimens immersed for four weeks had a greater amount of precipitate than at one week. High-magnification SEM photographs of the treated specimens immersed in SBF for four weeks are shown in Fig. 7. The surfaces of all treated specimens had precipitates with needle-like and island-like crystals. Island-like precipitates of the titanium treated in CaO solution at 200 °C were larger than those of the other treatments. Fig. 8 shows the XRD pattern of a specimen treated in CaO solution at 280 °C, after immersion in SBF for four weeks. The XRD pattern indicated that the precipitates on the titanium specimen were apatite crystals because of the bump peak range from 2θ 30° to 32° for the apatite crystal. Mass changes of the treated specimens before and after immersion in SBF for one and four weeks are shown in Fig. 9. There were no differences in mass change between the plates set vertically and that laid at the bottom. The mass of all specimens increased with immersion in SBF. However, the mass of non-treated titanium decreased slightly after immersion in SBF for four weeks. As for the treated specimens, their mass increased remarkably after immersion in SBF for four weeks as compared to their mass after one week's immersion (ANOVA, $p < 0.05$), except for the specimen treated in water at 200 °C ($p > 0.05$). In particular, mass of the specimen treated in CaO solution at 280 °C was remarkably increased compared with those of the other specimens. A correlation was thus estimated from the results of mass change measurement and SEM observation.

The mean tensile strength values of non-treated and treated specimens are shown in Table 3. No influence of hydrothermal treatment on the strength of titanium specimens was confirmed.

Table 2 Relation between oxygen (O), hydroxyl group (OH), and water (H₂O) contents on the surface layer of titanium specimens after hydrothermal treatment (XPS)

Outermost layer			OH/O	(H ₂ O+OH)/O
200°C	1.4 MPa	Water	0.46	0.51
280°C	6.3 MPa	Water	0.39	0.46
200°C	1.4 MPa	CaO solution	2.48	3.32
280°C	6.3 MPa	CaO solution	1.80	2.19

After sputtering			OH/O
200°C	1.4 MPa	Water	0.20
280°C	6.3 MPa	Water	0.21
200°C	1.4 MPa	CaO solution	0.36
280°C	6.3 MPa	CaO solution	0.29

Fig. 6 Scanning electron micrographs (SEM) showing the surface of titanium specimens with hydrothermal treatment, HT (A, B, C, D) and without HT (N) after immersion in SBF for 1 and 4 weeks.

Fig. 7 Scanning electron micrographs (SEM) showing the surface of titanium specimens with hydrothermal treatment (HT) after immersion in SBF for 4 weeks. A: HT at 200°C in water; B: HT at 280°C in water; C: HT at 200°C in CaO solution; and D: HT at 280°C in CaO solution.

Fig. 8 X-ray diffraction pattern of titanium specimen with hydrothermal treatment (HT) at 280°C in CaO solution after immersion in SBF for 4 weeks.

Fig. 9 Relationship between mass change and temperature of surface-modified titanium with immersion in SBF solution at 37°C for 1 and 4 weeks.

Table 3 Comparison of tensile strengths of titanium specimens after hydrothermal treatment (HT) in water and CaO solution

Treatment			Tensile strength, MPa
HT in Water	121°C	0.2 MPa	394 (1)
	200°C	1.4 MPa	395 (1)
	280°C	6.3 MPa	390 (3)
HT in CaO solution	121°C	0.2 MPa	385 (3)
	200°C	1.4 MPa	388 (3)
	280°C	6.3 MPa	385 (2)
Non-treated			389 (2)

(): Standard deviation

DISCUSSION

Surface modification of titanium due to hydrothermal treatments

High-pressure hydrothermal treatments are known to induce various reactions that do not occur at atmospheric pressure. We demonstrated that titanium specimens with hydrothermal treatment in calcium-containing solutions at 121°C (0.2 MPa) showed more effective bioactivity than those only immersed in calcium-containing solutions^{1,14}. The hydrothermal treatment used in this study applied a maximum temperature and pressure of 280°C and 6.3 MPa respectively, the high pressure being 60 times more than that of normal atmosphere.

The XRD pattern of non-treated titanium did not reveal the presence of titanium oxide because the oxide film of titanium was remarkably thin. However, the XRD patterns revealed that hydrothermal treatment in CaO solution increased the thickness of titanium oxide film as compared with that in water. Moreover, crystallization of calcium titanate (CaTiO_3) was confirmed by the XRD patterns of specimens treated in CaO solution. We have proved that calcium titanate on the surface of titanium had more effective bioactivity for osteointegration than anatase (TiO_2) or rutile (TiO_2) on the surface³². In our previous study, the crystallinity of calcium titanate was increased by hydrothermal treatment at 121°C and 0.2 MPa in CaO solution as compared with the case of mere immersion in the same solution¹. Therefore, even a hydrothermal treatment at low temperature and pressure (121°C and 0.2 MPa) used for sterilization could induce a reaction that did not occur under atmospheric pressure at 121°C. It was therefore hypothesized that the crystallinity of calcium titanate could be increased by increasing the pressure and temperature of hydrothermal treatments. The crystallinity of calcium titanate and exact thickness of the titanium oxide film on the titanium surface could not be estimated by XRD measurement because the crystal phase of the titanium surface in this study was not measured using thin-film XRD. However, XRD measurement implied that the thickness of titanium oxide film and calcium titanate film on the surface layer increased with increase in hydrothermal pressure (Fig. 5). Further, XPS measurement also revealed that the OH group content in the surface layer was increased by hydrothermal treatment (Table 2). OH groups on the surface of titanium are known to be effective for biocompatibility and bioactivity in osteointegration^{1,32}.

By physical estimation, it could be said that the mechanical properties of titanium specimens were reduced by hydrothermal treatments at high pressure because a surface layer was introduced on titanium — whereby thickness of oxide film increased with increase in pressure, and likewise the constituent types

in the surface layer. However, the surface layer introduced by hydrothermal treatments was very thin. Therefore, for titanium specimens with 1 mm diameter, their tensile strength was not affected.

Immersion in SBF for surface-modified titanium

In the evaluation of biomaterials such as bone substitutes, SBF is the *de facto* immersion solution used to reproduce *in vivo* surface structure changes^{33–35}. It is known that carbonated apatite is precipitated on the surfaces of materials by immersion in SBF because of its biocompatibility and bioactivity, that is, osteointegration and osteobonding. Evaluation is then performed by observing the precipitation time and volume of the apatite formed^{35,36}. In fact, since 10 years ago, we have been trying to adopt weight measurement for the estimation of precipitation volume with immersion in SBF^{1,14,35,37}.

For precipitation on the surfaces of specimens after immersion in SBF, there was a relation between SEM observation (Fig. 6) and weight measurement (Fig. 9). Results after immersion in SBF for four weeks showed that hydrothermal treatment in water was more effective for apatite precipitation on the titanium surface than no hydrothermal treatment. This was the reason why a large quantity of OH groups and a thick layer of TiO_2 appeared on the surface of titanium with hydrothermal treatment in water, as shown in Fig. 9. With hydrothermal treatment in CaO solution, precipitates on the titanium surface were larger than those of hydrothermal treatment in water and no hydrothermal treatment. One reason was because OH groups increased remarkably on the surface of titanium with hydrothermal treatment in CaO solution (Table 2). Moreover, in the surface layer of titanium with hydrothermal treatment in CaO solution, there was almost no TiO_2 because Ca(OH)_2 was adsorbed and calcium titanate (CaTiO_3) was introduced by the hydrothermal reaction (Fig. 5, Table 1). Calcium titanate on the surface of titanium had more effective bioactivity than anatase (TiO_2) and rutile (TiO_2)³⁴. CaO solution, *i.e.*, Ca(OH)_2 solution, has a high pH value and apatite precipitation might be increased by the high pH on the surface^{1,14}. Thus, hydrothermal treatment in CaO solution was most effective for apatite precipitation.

Results of this study suggested that hydrothermal treatments at 1.4 MPa and 6.3 MPa could be effective for surface modification of titanium as an implant material compared with hydrothermal treatment at 0.2 MPa and no hydrothermal treatment. Moreover, they revealed that hydrothermal treatment in CaO solution was better for the surface modification of titanium than in water. Better precipitation of bone-like apatite on surface-modified titanium will lead to rapid osteointegration with bone when implanted in the body as an artificial tooth

root. Leveraging on the results of this study, the clinical applicability and availability of high-pressure hydrothermal treatments — as a surface modification technique for titanium — should be further assessed in the future by evaluating osteointegration in *in vivo* animal experiments and *in vitro* cell cultures.

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