Surface Topography Designed to Provide Osteoconductivity to Titanium after Thermal Oxidation

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Hydroxyapatite formation on the surface of materials in the body is an essential condition for demonstrating osteoconductivity after implantation in bony defects. This paper reports a technique for providing hydroxyapatite formation properties to titanium metals by using specially designed surface topography followed by thermal oxidation. Two pieces of titanium thermally oxidized at 400°C were set together in a V-shape with varied mouth opening. They showed the formation of hydroxyapatite on both facing surfaces after exposure to a simulated body fluid (SBF), when the gap height was approximately less than 600 μm. Moreover, pure titanium specimens with macro-grooves less than 1000 μm in depth and 800 μm in width were able to form hydroxyapatite deposits in SBF within 604.8 ks, after they were thermally oxidized at 400°C for 3.6 ks. Hydroxyapatite also formed on the internal surfaces of macro-grooves made in Ti-15-Zr-4Ta-4Nb within 604.8 ks of soaking in SBF, after the sample was thermally oxidized at 500°C for 3.6 ks, whereas it was not deposited on alloy made of Ti-6Al-4V extra low interstitial metal processed in the same way. These findings indicate that titanium and its alloys can be conferred with hydroxyapatite-forming ability, i.e. osteoconductivity, within a controlled spatial gap and thermal oxidation. We conclude that bioactive titanium substrate showing osteoconductivity can be produced by using a specially designed surface topography followed by thermal oxidation at an appropriate temperature.

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1. Introduction

Titanium and its alloys have been widely used as artificial joints, fracture fixation devices, and dental implants, because of their good biological affinity to bone. The biological affinity of these metals is attributed to the passive film formed on their surfaces, i.e. formation of titanium oxides, which lead to high chemical durability. Among the titanium oxide compounds, titanium dioxide is regarded as a bioinert material that is stable in physiological conditions, but is intermediated by a thin fibrous layer at the interface between bone and metal when implanted in body defects.1) This means that titanium metal does not generally show a direct bond with bone, i.e. show osteoconductivity. We are aware that bioactive ceramics make a direct bond with living bone, when implanted in bony defects. Bioactivity of implant materials in bone usually implies that they are osteoconductive.

Previous studies revealed that the essential prerequisite for artificial materials to show osteoconductivity is formation of a bone-like hydroxyapatite layer on their surface after implantation in bony defects.2–4) To provide osteoconductivity to titanium metal, many researchers have proposed several chemical treatments including with NaOH solution5,6) or H2O2 solution.7,8) These treatments create Ti-OH groups in a titania hydrogel layer on the titanium surface, and it has been reported that Ti-OH provides a catalytic effect to trigger heterogeneous nucleation of hydroxyapatite in the simulated body fluid (SBF).9,10) SBF has ion concentrations almost equal to those in human blood plasma and is used to evaluate the potential for bone-like hydroxyapatite layer formation in vitro. Osaka and his colleagues reported enhancement of hydroxyapatite formation in SBF through a specific V-shape arrangement of thermally oxidized titanium specimens. In their reports,11,12) induction of hydroxyapatite deposition in SBF was observed on the surfaces of the internal space created when two pieces of titanium substrate were juxtaposed, and was bolstered by a piece of nylon wire at one edge. The hydroxyapatite deposition was only observed on the internal surfaces after soaking in SBF for 604.8 ks, but not on the external surfaces exposed to SBF, even when soaked for two months. The titanium specimens oxidized at 400°C or 500°C were shown to have the highest hydroxyapatite-forming ability on their internal surfaces. This finding led us to the novel idea that controlling both specific space and thermal oxidation temperature enhanced the potential for hydroxyapatite formation on titanium. If we could successfully apply this fundamental finding to the surface topography of titanium and titanium alloys, bioactive metallic materials would be produced through simple processing by means of a specially designed surface topography followed by thermal oxidation. Hence, we investigated the tendency for hydroxyapatite formation in spatial gaps of different distances between internal surfaces of pure titanium thermally oxidized at 400°C, in order to determine the optimum design of the groove. The surface topography design was then modified with macro-grooves, ranging from 200 to 1000 μm wide and deep, made on the surfaces of plates of pure titanium and titanium alloys followed by heat treatment at an appropriate temperature. Their hydroxyapatite-forming ability in SBF was examined to estimate their potential osteoconductivity.

2. Materials and Methods

2.1 Specimens

Pure titanium was purchased from Kobe Steel Ltd. and Ti-
6Al-4V extra low interstitial (ELI) from Sumitomo Metal Industries, Ltd. Ti-15Zr-4Ta-4Nb was kindly supplied by Dr. Okazaki, National Institute of Advanced Industrial Science and Technology (AIST), Japan.

To evaluate the effect of a spatial gap on the induction of hydroxyapatite formation in SBF, rectangular specimens of pure titanium were cut to $30 \times 10 \times 2 \text{ mm}^3$, and then gently washed with acetone and ultrapure water in an ultrasonic cleaner. The specimens were placed in muffle furnace to be thermally oxidized in air at 400°C for 3.6 ks.

To evaluate the effect of macro-grooves on the induction of hydroxyapatite formation in SBF, macro-grooves were machined to various depths and widths less than 1000 μm in $5 \times 5 \times 5 \text{ mm}^3$ specimens of the pure titanium, Ti-6Al-4V ELI, and Ti-15Zr-4Ta-4Nb. Subsequently, the pure titanium and titanium alloy specimens were thermally oxidized for 3.6 ks in air at 400°C and 500°C, respectively. The appearance of a pure titanium specimen with macro-grooves before thermal oxidation is shown in Fig. 1.

2.2 Evaluation of hydroxyapatite formation

Conditions for immersion of the specimens are shown schematically in Figure 2. To evaluate the effect of a spatial gap on the induction of hydroxyapatite formation in SBF, rectangular $30 \times 10 \times 2 \text{ mm}^3$ specimens of titanium plate were juxtaposed, bolstered with a piece of titanium wire of 0.1, 0.3, 1, 2, or 3 mm in diameter, as shown in Fig. 2(a). Specimens set up in this way were soaked in 30 cm³ of SBF (Na⁺ 142.0, K⁺ 5.0, Mg²⁺ 1.5, Ca²⁺ 2.5, Cl⁻ 147.8, HCO₃⁻ 4.2, HPO₄²⁻ 1.0, and SO₄²⁻ 0.5 (concentrations in mmol/dm³), pH 7.40 at 36.5°C) for predetermined periods up to 604.8 ks without stirring. Specimens with macro-grooves were immersed in 30 cm³ of SBF for 604.8 ks without stirring. The machined surface of the specimens was placed face down on the bottom of a flat-bottomed polystyrene bottle, as shown in Fig. 2(b). After soaking in SBF for 604.8 ks, the specimens were removed from the solution, gently washed with ultrapure water and then dried in air. The internal surface of the rectangular specimens was observed both by the naked eye and by scanning electron microscopy (SEM, JSM-6300, JEOL Ltd., Tokyo, Japan) after coating of thin film of gold on its surface by sputtering. In the visual inspection, hydroxyapatite deposition was determined by a change in regions of the surface to a white cloudy appearance. The ability for hydroxyapatite formation was regarded as the distance of hydroxyapatite deposition from the closed end of the V-shaped double rectangular specimens separated at the other end by a titanium wire, determined using digital photographs and image analysis software (ImageJ, National Institutes of Health, Bethesda, MD, USA).

For evaluation of the hydroxyapatite formation on the internal surface of the macro-grooves in specimens after soaking in SBF, the deposition of particles was regarded as formation of hydroxyapatite, because deposited particles on chemically treated titanium in SBF has already been recognized as formation of bone-like hydroxyapatite. Observation of the surface in the macro-grooves was performed using a field-emission scanning electron microscope (FE-SEM, S-4700, Hitachi Co., Ltd., Tokyo, Japan and JSM-7500FA, JEOL Ltd.), equipped with energy dispersive X-ray microanalyzer (EDX, JED-2300, JEOL Ltd.).

2.2.1 Evaluation of hydroxyapatite formation

Fig. 1 Appearance of a pure titanium specimen with macro-grooves before thermal oxidation.

Fig. 2 Schematic illustrations of specimens soaking in SBF. (a) Rectangular specimens juxtaposed and bolstered with pieces of titanium wire of various diameters at one edge to create a gap (bolstering wire: 0.1, 0.3, 1, 2 and 3 mm, X: distance from the lower end in height, Z: height of internal gap). (b) Cubic specimens with various macro-groove sizes are placed face down on the flat bottom of a container (D: depth, W: width (X = 1, 2, 3, 4)).
3. Results

3.1 Hydroxyapatite-forming ability of the V-shaped specimens

Figure 3 shows the appearance of the internal surfaces of the V-shaped specimens established with a spatial gap by using the various sizes of titanium wire, after soaking in SBF for the predetermined periods. There was no remarkable change on the pure titanium specimen without heat treatment after soaking in SBF, even when the spatial gap was established with a wire of 0.3 mm in diameter. In contrast, a change to a white cloudy appearance was observed on both the internal surfaces for specimens heated at 400°C, after soaking in SBF. When comparing the area on the internal surfaces of titanium samples heated at 400°C, after soaking in SBF, a smaller gap established with titanium wire resulted in a larger area of hydroxyapatite formation after soaking in SBF for 432.0 ks. Namely, the area of apatite formation increased with decreasing gap height in order of the wire diameters: 3 mm < 2 mm < 1 mm < 0.3 mm < 0.1 mm. Figure 4 shows a SEM photograph of the internal surface of a pure titanium specimen thermally oxidized at 400°C, after soaking in SBF for 259.2 ks, where the specimens were bolstered using 0.3 mm diameter titanium wire. An assembly of fine particles almost completely covered the internal surface of the specimen after exposure to SBF for 259.2 ks. As seen in Figure 4(b), the morphology of the particles is quite similar to that of hydroxyapatite formed on bioactive glasses and glass-ceramics after soaking in SBF, according to a previous report. Hence, the surface change

<table>
<thead>
<tr>
<th>Diameter of bolstered wire, $D_1 / \text{mm}$</th>
<th>Treatment</th>
<th>Soaking period, $T / \text{ks}$</th>
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<tr>
<td></td>
<td></td>
<td>86.4</td>
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<tr>
<td>0.3</td>
<td>Non-treatment</td>
<td>259.2</td>
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<tr>
<td>0.1</td>
<td>400 °C, 3.6 ks</td>
<td>432.0</td>
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<tr>
<td>0.3</td>
<td>400 °C, 3.6 ks</td>
<td>604.8</td>
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<tr>
<td>1.0</td>
<td>400 °C, 3.6 ks</td>
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<tr>
<td>2.0</td>
<td>400 °C, 3.6 ks</td>
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<td>3.0</td>
<td>400 °C, 3.6 ks</td>
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Fig. 3 Macroscopic observation of the internal surfaces of specimens with a spatial gap established by using the various sizes of titanium wire, after soaking in SBF for predetermined periods (Bar: 5 mm).

Fig. 4 SEM photographs of the internal surfaces between the titanium substrates after soaking in SBF for 259.2 ks, when the specimens were bolstered using 0.3 mm diameter titanium wire.
to a white cloudy appearance on macroscopic observation is attributed to deposition of the hydroxyapatite. From these observations, the tendency of the hydroxyapatite formation is summarized in Table 1, with relationship of the maximum height of the gap, which was calculated from the geometric relationship of the length of the area in which hydroxyapatite was deposited. The height of the gap at the point up to where the hydroxyapatite was deposited on the internal surface of the specimens after soaking in SBF for 432.0 ks was approximately equal to that for 604.8 ks. This tendency was not dependent on the diameter of the titanium wire. These findings indicated that the hydroxyapatite deposition was almost complete within 432.0 ks soaking in SBF. Note that hydroxyapatite deposition was observed on an area up to less than a gap height of 630 ± 127 μm, irrespective of the diameter of the titanium wire.

Table 1 Relationship between gap length and height of the area of the hydroxyapatite formation in SBF. Maximum gap height was estimated at the point marking the extent of the hydroxyapatite formation from the edge where the rectangular specimens were in contact.

<table>
<thead>
<tr>
<th>Diameter of bolstered wire, $D_1$/mm</th>
<th>Soaking period, $T$/ks</th>
<th>Length of the area on hydroxyapatite formation*, $L$/mm</th>
<th>Estimated height of gap**, $H$/μm</th>
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<tbody>
<tr>
<td>1</td>
<td>432.0</td>
<td>17.1</td>
<td>571.7</td>
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<tr>
<td>1</td>
<td>604.8</td>
<td>17.7</td>
<td>589.1</td>
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<tr>
<td>2</td>
<td>432.0</td>
<td>12.5</td>
<td>833.0</td>
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<tr>
<td>2</td>
<td>604.8</td>
<td>7.2</td>
<td>485.9</td>
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<tr>
<td>3</td>
<td>432.0</td>
<td>5.7</td>
<td>574.3</td>
</tr>
<tr>
<td>3</td>
<td>604.8</td>
<td>7.3</td>
<td>730.9</td>
</tr>
</tbody>
</table>

*Hydroxyapatite formation implies the distance from the lower end in height, when the surfaces of the specimens were changed the color to white.

**Estimated height of gap was calculated from the value of the distance of hydroxyapatite formation.

3.2 Hydroxyapatite formation in the macro-grooves

Figure 5 shows SEM images of the internal surfaces of macro-grooves (500 μm deep and wide) machined in the pure titanium specimens, after soaking in SBF for 604.8 ks. (a) and (b), Macro-grooves were placed facing downward (toward the bottom of the bottle); (c) and (d), macro-grooves were facing upward (toward the top of the bottle). (a) and (c), Specimens with thermal oxidation at 400°C; while (b) and (d) were without the thermal oxidation.

Table 1

<table>
<thead>
<tr>
<th>Diameter of bolstered wire, $D_1$/mm</th>
<th>Soaking period, $T$/ks</th>
<th>Length of the area on hydroxyapatite formation*, $L$/mm</th>
<th>Estimated height of gap**, $H$/μm</th>
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formation of such deposits even after 604.8 ks, irrespective of thermal treatment (Figs. 5(c) and (d)). In summary, the apatite deposition occurred in SBF when the grooves were placed downward on the flat-bottomed surface of their container after the sample was heat-treated at 400°C. The closed area facing the bottom of the container was effective in inducing the heterogeneous nucleation of hydroxyapatite.

Figure 6 shows FE-SEM images of and EDX spectra from the internal surface of a macro-groove 200 μm deep and 500 μm wide made on pure titanium substrate, thermally oxidized at 400°C, after soaking in SBF facing downward (toward the bottom of the container) for 604.8 ks. The morphology of the hemispherical particles is similar to that of hydroxyapatite deposited on the surface of the internal surface established in the spatial gaps, as seen in Fig. 4. In the EDX results, peaks assigned to calcium and phosphate were clearly detected in the deposited particles. These findings support the hypothesis that the deposited particles have hydroxyapatite characteristics. The tendency for hydroxyapatite formation in the macro-grooves made in titanium samples subsequently heat-treated at 400°C is summarized in Table 2. After the exposure of macro-grooves with various depths and widths to SBF with samples facing downward, the internal surface of the grooves were observed under SEM to determine the formation of hydroxyapatite. As seen in Table 2, the grooves both less than 800 μm wide and 1000 μm deep allow the formation of hydroxyapatite in the grooves. Figure 7 shows SEM photographs of the Ti-6Al-4V ELI and Ti-15Zr-4Ta-4Nb samples with macro-grooves 500 μm wide and 500 μm deep, which were thermally oxidized at 500°C for 3.6 ks, and then soaked in SBF in a downward direction for 604.8 ks. It is clear that particles of hydroxyapatite were deposited on the internal surface of macro-grooves in heat-treated Ti-15Zr-4Ta-4Nb specimens after soaking in SBF for 604.8 ks. In contrast, the internal surface of the macro-grooves in heat-treated Ti-6Al-4V ELI samples did not have hydroxyapatite deposits even after soaking in SBF for 604.8 ks.

4. Discussion

The results clearly show that a gap of less than 600 μm high induced the heterogeneous nucleation and crystal growth of hydroxyapatite on titanium metal in SBF, when the titanium substrate had been thermally oxidized at 400°C. The pure titanium specimens with macro-grooves less than 1000 μm deep and 800 μm wide also formed hydroxyapatite on their internal surfaces, when samples were placed in SBF facing downward toward the flat bottom of their container. It is clear from Figure 3 that the hydroxyapatite-forming ability on thermally oxidized pure titanium with a spatial gap is influenced by two factors: One factor is the characteristics of a passive film on the surface of the titanium, implying a crystalline phase and a number of hydroxyl groups, which is changed by thermal oxidation. The other is the height of the gap, which includes the volume and/or opening of the reaction space. The oxidation of the substrate surface through heat treatment at around 400°C allows the formation of crystalline rutile-phase titanium dioxide, which is about 30 nm thick.11,12,18) While specimens thermally oxidized at less than 300°C were covered by microcrystalline and/or amorphous titanium dioxide on its surface. In general, the number of hydroxyl groups on the surface of inorganic
Therefore, surfaces of pure titanium thermally oxidized at 400°C would be facilitated not only by the amount of Ti-OH groups, but also by its crystalline planar arrangement.\textsuperscript{19,20)} Anatase would be induced by functional groups on the surface of rutile and hydroxyapatite on the surface of rutile and anatase would be facilitated not only by the amount of Ti-OH groups, but also by its crystalline planar arrangement.\textsuperscript{19,20)}

Previous reports have suggested that heterogeneous nucleation of hydroxyapatite on the surface of rutile and anatase would be facilitated not only by the amount of Ti-OH groups, but also by its crystalline planar arrangement.\textsuperscript{19,20)}

Thus, there may be effects from the surface charges of the titanium dioxides. These phenomena are very complicated and the details have to be clarified by future work.

The Ti-15Zr-4Ta-4Nb specimens thermally oxidized at 500°C were set together in a V-shape with varied mouth opening, and they formed hydroxyapatite on both internally facing surfaces in SBF, between the open mouth spaces of up to approximately 600 μm. Hydroxyapatite formation on the internal surfaces of macro-grooves in pure titanium thermally oxidized at 400°C and in Ti-15Zr-4Ta-4Nb thermally oxidized at 500°C could be induced within 604.8 ks in SBF. This spatial design provides a new technique for conferring bioactivity to titanium by using only machining and thermal oxidation, and is a new concept for bioactive titanium-based metals.

5. Conclusions

Hydroxyapatite formation on the titanium alloys might be inhibited by metallic elements such as Al. In this report, we applied 500°C for surface oxidation of titanium alloys after preliminary examination found oxidation around a range from 400 to 600°C.

Consequently, this type of technique straightforwardly confers bioactivity, i.e. osteoconductivity on titanium and its alloys through the design of surface topography and subsequent heat treatment with appropriate oxidation conditions. The size of gaps allowing the expression of bioactivity, around 100–500 μm, are also effective for bone ingrowth on artificial prostheses. Therefore, this type of simple technique, i.e. “spatial design”, can make bioactive titanium implants strongly osteoconductive, as well as providing a new concept for bioactive titanium-based metals.

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