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Amount of hydroxyl radical on calcium-ion-implanted titanium and point of zero charge of constituent oxide of the surface-modified layer

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To compare the surface properties of calcium-ion (Ca^{2+})-implanted titanium with those of titanium and to investigate the mechanism of bone conductivity of Ca^{2+}-implanted titanium, amounts of hydroxyl radical of Ca^{2+}-implanted titanium and titanium were estimated. Also, the point of zero charge (p.z.c.) of oxide constituting surface oxides of Ca^{2+}-implanted titanium and titanium was determined. Results showed that the amount of active hydroxyl radical on Ca^{2+}-implanted titanium was found to be significantly larger than that on titanium, indicating that the number of electric-charging sites of Ca^{2+}-implanted titanium in electrolyte is more than that of titanium. The p.z.c. values of rutile (TiO_2), anatase (TiO_2), and perovskite ($CaTiO_3$), were estimated to be 4.6, 5.9, and 8.1, respectively. Thus, Ca^{2+}-implanted titanium surface is charged more positively in bioliquid than titanium, accelerating the adsorption of phosphate ions. © *1998 Chapman & Hall*

1. Introduction

Recently, the authors found that calcium phosphate precipitation on titanium in an electrolyte is markedly accelerated by calcium-ion (Ca^{2+})-implantation with 18 keV [1]. This phenomenon is also observed in Ca^{2+}-implanted titanium on which osteogenic cells are incubated [2]. In addition, osteogenic cells on titanium are activated and the formation of osteoid tissue by the cells is accelerated when calcium ions are implanted in titanium. A large amount of new bone was formed early on the Ca^{2+}-implanted titanium, compared with titanium, even at 2 days after implantation into rat tibia [3]. Therefore, Ca^{2+}-implanted titanium has interesting properties and should be useful as a biomaterial. The surface oxide of titanium grows as a result of the ion implantation and implanted calcium ions exist in the oxide as calcium titanate and/or calcium oxide [4, 5]. This surface-modified layer works as a substrate improving bone conduction. The surface properties of Ca^{2+}-implanted titanium should be investigated to elucidate the cause of the improved bone conductivity.

One of the causes of improved bone conductivity of calcium-ion-implanted titanium may be possibly microdissolution of calcium ions into bioliquid, inducing the precipitation of calcium phosphate on the material by supersaturation in the bioliquid near the material. Recently, the present authors revealed the microdissolution of calcium into aqueous solutions using X-ray photoelectron spectroscopy (XPS) [6] and Auger electron spectroscopy [7]. Oxygen decreases and titanium increases in the surface oxide according to the dissolution. In addition, hydroxyls radical decrease in number per unit area and the proportion of Ti^{4+} in titanium increases. Core-level binding energies of calcium and titanium originating from Ti^{4+} decreases with the dissolution. These changes are caused by the transformation of surface oxide according to the dissolution. That is, calcium dissolves and, consequently, the surface oxide becomes titanium oxide.

The electric charge on the surface is another important cause governing the hard tissue compatibility. Therefore, factors determining the electric charge also need to be characterized. In this study, the amounts of active hydroxyl radical and point of zero charge (p.z.c.) were estimated. The number of hydroxyl radicals was compared between Ca^{2+}-implanted and

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unimplanted titanium. The number corresponds to the number of electric-charging sites on the surface in the electrolyte. The p.z.c. is the pH value at which the electric charge of the surface is balanced. That is, it is a kind of indicator of the magnitude of positive or negative charge at the surface in a bioliquid. However, the estimation of p.z.c. values of Ca^{2+}-implanted and unimplanted titanium is difficult because a large surface area is needed. Therefore, the p.z.c. of perovskite ($CaTiO_3$) and titanium dioxide (TiO_2: rutile and anatase) powders were determined. The oxides are components of the surface oxide layers of Ca^{2+}-implanted and unimplanted titanium. The causes of improved hard tissue compatibility of Ca^{2+}-implanted titanium are discussed on the basis of the results. This information will enhance our understanding of the mechanism of bone conduction around biomaterials.

2. Materials and methods

2.1. Preparation of materials

Commercially pure titanium (ASTM grade 1) plates were metallographically polished with SiC paper and finally polished with 1-μm diamond paste. The plates were ultrasonically washed in acetone and ethyl alcohol for 15 min each. Calcium ions were implanted in the titanium with an acceleration energy of 18 keV and an ion-beam current-density of 50 $\mu A\,cm^{-2}$. The assist gas for the implantation was krypton. The pressure in the chamber for the ion implantation was approximately 10^{-4} Pa. Calcium ions were uniformly implanted at 10^{18} ions cm^{-2}. The surface-modified layer of the Ca^{2+}-implanted titanium consists of calcium titanate and calcium oxide [5]. There is a possibility that the outermost surface of the surface-modified layer is calcium hydroxide in both cases described above. Calcium ions exist in the surface oxide which grows with ion implantation. The thicknesses of the surface modified layers are very thin: approximately 13 nm [4]. After the implantation, the specimens were washed and dried by the method described above. The sizes of specimens were 10 × 10 × 1 mm.

Three kinds of commercial oxide powders were used for the estimation of p.z.c.: TiO_2 (rutile and anatase, Wako Pure Chemical Industries, Osaka, Japan) and $CaTiO_3$ perovskite, (High Pure Chemicals, Sakado, Saitama, Japan). The mean particle sizes were determined to be 0.28 μm for rutile, 1.27 μm for anatase, and 1.20 μm for perovskite using a particle size analyser (CAPR-300, Horiba, Tokyo, Japan). Five grams of powder was used for the experiments. The exact surface area of oxide powder could not be determined precisely because the powder had a distribution in the particle size, but was estimated to be approximately 8.32 m^2 for rutile, 6.15 m^2 for anatase, and 6.23 m^2 for perovskite.

2.2. Amount of hydroxyl radical

Amounts of hydroxyl radical existing on Ca^{2+}-implanted titanium and titanium surfaces in air were determined by the following methods. Ammonium chloride solution (4 $mol\,l^{-1}$ and 500 ml) was mixed with zinc chloride (0.4 $mol\,l^{-1}$ and 250 ml) solution and the pH of the mixture was adjusted with 30% ammonium hydroxide to 6.9. The volume of the mixture was adjusted to 1000 ml with deionized water.

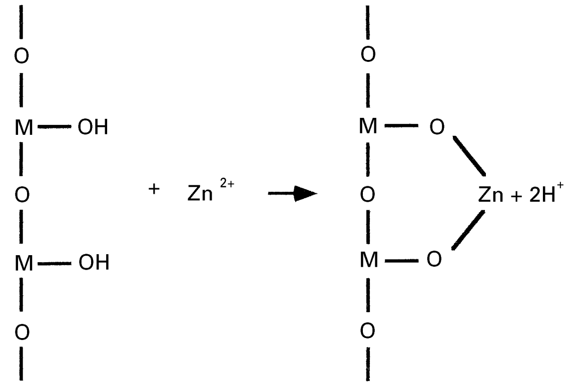

Figure 1 Formation of zinc chelate by the reaction of active hydroxyl radicals (OH) on the surface of a metal (M) oxide with a zinc chloride reagent.

Calcium-ion-implanted titanium and titanium were immersed one by one in 150 ml of the above resultant solution in beakers at ambient temperature for 300 s. During immersion, active hydroxyl radicals were mostly exchanged for zinc ions and zinc chelates were formed [8] as shown in Fig. 1. After this, the plates were immersed in 150 ml of deionized water three times to remove chemical species without adsorption. The specimens were dried in a desiccator and then immersed in 90 ml of 2.42 $mol\,l^{-1}$ nitric acid for 600 s to release zinc ions into the nitric acid solution. The volume of the solution containing zinc ions was adjusted to 100 ml with deionized water.

The amount of zinc ions in the nitric acid solution was determined using an inductively coupled plasma atomic emission spectrometer (ICP-AES: PS-3000UV, Leeman Labs, Inc., Lowell, MA). The zinc concentration corresponds to the amount of active hydroxyl radicals on the surface of a specimen. That is, double the estimated amount of zinc per unit of surface area of the plate equals the number of active hydroxyl radicals. Three specimens were used for each condition. The method is described in detail elsewhere [8, 9].

2.3. Point of zero charge

The point of zero charge of oxides was determined according to Wakamatsu and Mukai [10]. Rutile, anatase, and perovskite powders were used as oxide powders. Nitric acid (0.1 $mol\,l^{-1}$) with and without 5 g of oxide powder was titrated with 0.1 $mol\,l^{-1}$ sodium hydroxide. The titration curve changed when oxide powder was added and an intersection of both curves was apparent. The pH value of this intersection equals the p.z.c. of the oxide. Fig. 2 may assist in the understanding of the determination of p.z.c. Three experiments were performed for each oxide powder.

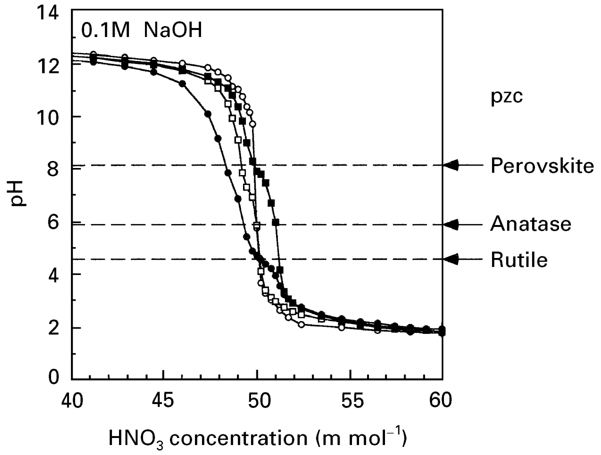

Figure 2 Titration curves of 0.1mol l^{-1} NaOH solution with and without (○) oxide powders (rutile (●), anatase (□) or perovskite (■)) by 0.1 mol l^{-1} HNO_3.

3. Results and discussion

3.1. Amounts of hydroxyl radicals on the surface

Estimated amounts per unit area of active hydroxyl radicals on Ca^{2+}-implanted titanium and titanium are shown in Fig. 3. The results in Fig. 3 are in an appropriate order because the amount for TiO_2 is in the range of 4.9–12.5 per nm^2 in reported references [11–13]. In any case, the amount of hydroxyl radical on Ca^{2+}-implanted titanium was significantly larger than that on titanium ($p < 0.01$). This result is supported by a previous XPS analysis showing that the proportion of hydroxyl radicals in total oxygen atoms is much larger in Ca^{2+}-implanted titanium than in titanium [4–6]. Calcium oxide and calcium titanate are covered by calcium hydroxide in air at the outermost surface because calcium hydroxide forms easily, inducing more hydroxyl radicals on Ca^{2+}-implanted titanium than on titanium. This is supported by a previous report that hydroxyl radicals decrease in number with a decrease of calcium oxide and calcium titanate and transform to titanium oxide by the dissolution of calcium [6].

Hydroxyl radicals on the surface dissociate in aqueous solutions and are electrically charged. This indicates that the number of electric-charging sites on Ca^{2+}-implanted titanium in aqueous solution is greater than that on titanium.

3.2. Point of zero charge

The titration curves with and without oxide powders are given in Fig. 2. The pH values of the intersection (i.e. the p.z.c. of oxides) were estimated to be 4.6 ± 0.4 for rutile, 5.9 ± 0.2 for anatase, and 8.1 ± 0.2 for perovskite. The difference between the values was significant ($p < 0.01$). These values are not completely accurate because the p.z.c. obtained by this method changes according to the ionic strength of the titrate and titrant [10]. Published p.z.c. values are 5.3 for rutile and 6.2 for anatase [14]. Therefore, the magnitude of p.z.c. obtained in this study is relatively correct and the p.z.c. of perovskite is larger than that of anatase, indicating that the surface of perovskite is charged more positively in aqueous solution than that of anatase. The titanium surface is covered by anatase and amorphous titanium dioxide [15] while the Ca^{2+}-implanted titanium surface is covered by calcium titanate [4, 5]. Strictly speaking, the oxide powders are different from surface oxides of Ca^{2+}-implanted titanium and titanium. However, the results for the powders are indicators of the surface properties of Ca^{2+}-implanted titanium and titanium.

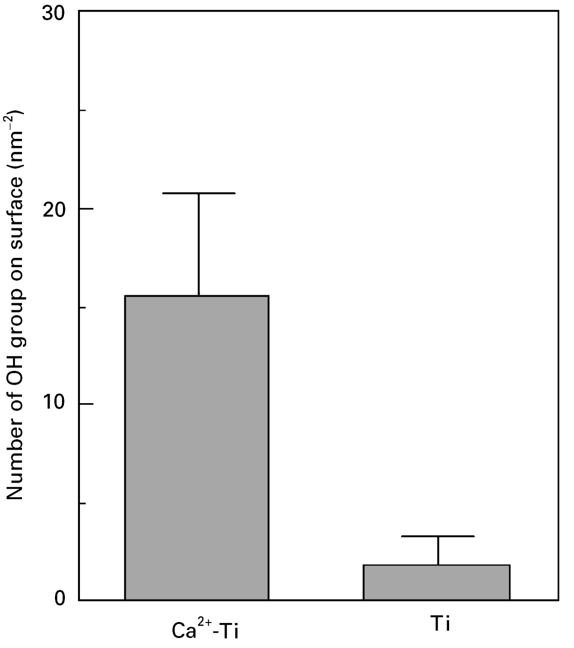

Figure 3 Amounts of active hydroxyl radicals on Ca^{2+}-implanted titanium (Ca^{2+}-Ti) and titanium per unit surface area.

Therefore, the surface of Ca^{2+}-implanted titanium is more positively charged than that of titanium in bioliquid. Such a surface facilitates the adsorption of hydroxyl radicals and phosphate ions in bioliquid because of the attractive force caused by electric charge. However, other anions are hardly adsorbed by the positive-charging sites; only hydroxyl radicals and phosphate ions are specifically adsorbed by Ca^{2+}-implanted titanium and titanium [1, 16, 17].

3.3. Behaviour of Ca^{2+}-implanted titanium in hard tissue

The Ca^{2+}-implanted titanium surface was more positively charged by dissociation of hydroxyl radicals than the titanium surface. In addition, the number of charging sites of Ca^{2+}-implanted titanium was greater than that of titanium. Adsorption of phosphate ions in bioliquid is greater on the Ca^{2+}-implanted titanium surface than on the titanium surface because of the attractive force of the electric charge. Greater adsorption of phosphate ions causes greater attraction of calcium ions by the surface, and more calcium phosphate precipitates. More proteins may be adsorbed by Ca^{2+}-implanted titanium than by titanium because of the larger number of charging sites.

Simultaneously, calcium ions dissolve from the surface-modified layer of Ca^{2+}-implanted titanium, as revealed in previous studies [6, 7]. This causes supersaturation for calcium phosphate precipitation in the bioliquid near the vicinity of the surface, and acceleration of calcium phosphate precipitation. The dissolution of calcium ions is faster in acidic solution than in neutral solution. When Ca^{2+}-implanted titanium is inserted in hard tissue, the pH around the material decreases to approximately 5.2 because of inflammation [18]. That is, more calcium ions are dissolved in the initial stage of insertion of Ca^{2+}-implanted titanium in hard tissue.

Characterization of surface properties is a useful method for the elucidation of the mechanism of biocompatibility of a biomaterial because the surface properties govern the interaction of the material and bioliquid. The interaction of an implant material with bioliquid is the first stage of the reaction determining biocompatibility.

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