Crystallization of amorphous titania gel by hot water aging and induction of in vitro apatite formation by crystallized titania

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Received 20 November 2005; accepted in revised form 17 December 2005
Available online 3 February 2006

Abstract

Surface modification of titanium implants by introducing a titania layer on their surface is an effective approach to provide bioinert titanium with bioactivity, i.e., the ability to bond directly and tightly to the surrounding hard tissue through the formation of a thin layer of apatite after implantation in the human body. The crystalline structure and abundance of Ti–OH functional groups have been found to contribute to the ability of titania gel to initiate apatite deposition on titanium in human physiological fluid. In the current investigation, an amorphous titania gel was firstly introduced on titanium surface by oxidizing the titanium substrate with hydrogen peroxide. Well-crystallized anatase films incorporated with abundant Ti–OH groups were then produced simply through a subsequent hot water aging of the amorphous titania gel. Results obtained in this investigation suggested that the low-temperature crystallization of titania proceeded in a dissolution–precipitation process. Titanium treated by the present low-temperature chemical modification technique induced significant apatite deposition within 24 h in a simulated body fluid (SBF).

Keywords: Titanium; Apatite; Bioactivity; Hydrogen peroxide; Low-temperature crystallization

1. Introduction

Commercially pure titanium (CPTi) is now widely used for hard tissue replacement due to its biocompatibility, excellent corrosion resistance, good mechanical properties and long fatigue life. In orthopaedics and dentistry, it is desirable for CPTi implants to be fixed tightly and reliably as quickly as possible after their implantation [1,2]. Hench et al. found that certain biomaterials can bond directly to surrounding tissues at the molecular level through the formation of a thin apatite layer on their surfaces [3]. Therefore, various physical, chemical, and electrochemical methods have been investigated in order to provide CPTi with the ability to induce apatite formation in human physiological fluid [4]. Among these methods, chemical modification of CPTi surface has attracted much attention due to its simplicity, effectiveness and being applicable to implants with complicated shapes [2,5–11]. A layer of titania, which induces apatite deposition in a simulated body fluid (SBF), can be produced on CPTi surface by treating the surface with either hydrogen peroxide [2,5–7] or sodium hydroxide solutions [8–11]. Through intensive investigations into mechanisms of apatite deposition on titania, it is now widely accepted that both Ti–OH functional groups and a crystalline titania, either as anatase or as rutile, contribute to fast apatite deposition [7,12–15]. To further improve the apatite deposition ability of CPTi, it is of great interest to employ low-temperature processes for the crystallization of titania, thus avoiding the commonly used thermal treatment, which causes the loss of beneficial Ti–OH functional groups, to crystallize amorphous titania gel.

A titania film consisting of a mixture of anatase and rutile could be produced on CPTi surface at a low temperature of 80 °C, using hydrogen peroxide solution containing tantalum chloride [7]. In a previous publication, it was shown that the
amorphous titania obtained by soaking CPTi in 30 mass% H₂O₂ solution at 80 °C for 8 h could crystallize into pure anatase by soaking in distilled water at 80 °C for 72 h [16]. This paper presents the results of further studies of the low-temperature crystallization process of titania (anatase) and the ability of the anatase film to induce apatite deposition in vitro.

2. Materials and methods

CPTi plates of the dimensions 10×10×0.1 mm³ were soaked at 60 °C for 2 min in a 1:3:6 mixture (in volume) of 55% HF, 63% HNO₃ and distilled water (DW). They were then ultrasonically cleaned in DW three times for a total duration of 15 min. The samples were subsequently immersed in 30 mass% H₂O₂ solution, with each sample being in 10 ml H₂O₂ solution in a polyethylene bottle (25 mm in diameter) with a tight screw cap, and kept at 80 °C in an oven for 2, 8, and 72 h. Aging of the samples was conducted by soaking each sample in 10 ml DW at 80 °C for 72 h. After aging, all samples were rinsed gently with DW and dried overnight at ambient temperature for further investigation.

The ability of the chemically modified titanium to induce in vitro apatite deposition was assessed by soaking each sample in 15 ml SBF at 36.5 °C for various durations. SBF was prepared by dissolving appropriate amounts of NaCl, NaHCO₃, Na₂CO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂, Na₂SO₄ and HCl in DW to give ion concentrations similar to those in human blood plasma [17]. The pH value of SBF was adjusted to 7.4 at 36.5 °C using trishydroxymethylaminomethane and 1 M HCl solution. Samples for SBF-soaking experiments were subjected to ultrasonic cleaning for removing any loose surface layer.

The surface morphology of samples was observed using a LEO 1530 field emission scanning electron microscope (FE-SEM). Thin-film X-ray diffraction (TF-XRD) patterns were recorded using a Rigaku RAD II A X-ray diffractometer with a thin-film attachment, which was operated with CuKα radiation at 40 kV–25 mA and at a scanning rate of 0.3° min⁻¹. Fourier transform infrared reflection (FT-IR) was performed using a JASCO FT-IR300 spectrometer. Differential scanning calorimetry (DSC) analyses were conducted using a Perkin Elmer Pyris 6 DSC, with a heating rate of 10 K min⁻¹ and under an atmosphere of Ar.

3. Results

Figs. 1–3 show surface morphologies of CPTi after soaking in the H₂O₂ solution at 80 °C for 2, 8 and 72 h, before and after hot water aging. Oxidizing CPTi for 2 h in the H₂O₂ solution resulted in a porous titania gel layer with cracks on the surface (Fig. 1a). The wall of the pores appeared to be smooth at a high magnification (×40,000) (Fig. 1b). After hot water aging, cracks disappeared (Fig. 1c), the porous network collapsed, and the layer consisted mainly of tiny particles having sizes of tens of nanometers (Fig. 1d). Similar morphological changes were
found for CPTi subjected to 8 h soaking in the H₂O₂ solution and also subsequent 72 h hot water aging (Fig. 2). After 72 h soaking in the H₂O₂ solution, a whisker-like titania layer covered thoroughly the pre-formed porous surface (Fig. 3a,b). After hot water aging, the whisker-like layer was replaced by a layer consisting of titania nano-rods, which were seen to be aggregates of tiny titania nanoparticles which aligned themselves to form the nano-rods (Fig. 3c,d). It was noted that the titania layer formed through 72 h soaking in the H₂O₂ solution was too thick to adhere to the substrate. It detached from the substrate during subsequent ultrasonic cleaning and only a thin layer consisting of condensed titania nanoparticles remained on CPTi surface, as shown in Fig. 4.

Fig. 5a displays the TF-XRD patterns of CPTi oxidized by the H₂O₂ solution at 80 °C for various times. Those of the oxidized CPTi followed by a hot water aging at 80 °C for 72 h are indicated in Fig. 5b. Without the subsequent hot water aging, only broad peaks at around 25.3° and 48.5° in 2θ, which correspond to titania with a mainly amorphous structure [16], can be identified for all the 3 samples. Note also in Fig. 5a the decreased intensities of peaks corresponding to the Ti substrate with increasing oxidizing time in the H₂O₂ solution, due to the increased thickness of the amorphous layer. After the hot water aging, peaks corresponding to well-crystallized anatase were found for all samples. The intensity of anatase peaks increased with increasing soaking time in the H₂O₂ solution, due to the increased thickness of anatase layer.

Titania powders detached from the CPTi surface oxidized by H₂O₂ solution at 80 °C for 72 h were collected for DSC analysis and a typical DSC curve of the powders is shown in Fig. 6. An endothermic peak located at ~185 °C and a relatively weak exothermic peak located at ~350 °C were observed. Molecurally chemisorbed water, which was non-dissociatively coordinated to Ti cations, was reported to desorb at 110 °C, 250 °C, or in the temperature range of 250 to 320 °C, depending on the initial water coverage [18]. Therefore, the endothermic peak observed in the current investigation is believed to be due to the removal of water molecules originally attached chemically to titania crystals.

XRD experiments indicated that the amorphous titania gel obtained by oxidizing CPTi with the H₂O₂ solution at 80 °C for 72 h crystallized to anatase at a temperature of 300 °C; therefore, the weak exothermic peak around 350 °C in Fig. 6 is unlikely to arise from the crystallization of titania. It is not clear at present time why this peak occurred.

The amorphous titania gel derived by oxidizing CPTi with the H₂O₂ solution at 80 °C for 2, 8, and 72 h failed to induce apatite deposition after subsequent soaking in SBF for one week. However, after the subsequent hot water aging to crystallize the titania gel to anatase, apatite deposition on treated samples was observed. Fig. 7 shows FT-IR spectra of CPTi oxidized by H₂O₂ solution at 80 °C for 8 h and aged in hot water at 80 °C for 72 h, followed by SBF-soaking for various times. The corresponding TF-XRD patterns of the
samples are displayed in Fig. 8. After soaking in SBF for 24 or 48 h, FT-IR spectra showed clearly bands for functional groups of carbonated hydroxyapatite, which included the peaks corresponding to phosphate (1115, 1085, 955, 603 and 564 cm$^{-1}$) and carbonate (1500, 1417 and 869 cm$^{-1}$) groups [19]. The broad peaks at $\sim$26° and 32° appearing in the TF-XRD patterns shown in Fig. 8 corresponded to apatite with poor crystallinity [20].

Fig. 9a,b are SEM micrographs of apatite particles having sizes of $\sim$1 μm which were found to cover the anatase film after the samples had been soaked in SBF for 24 and 48 h, respectively. A careful examination of the apatite layer revealed that each spherical apatite particles consisted of a large number of tiny flakes (Fig. 9a), which is characteristic of apatite particles formed in SBF on various substrates including titania gel [4–15], collagen membrane [21], bioactive glass or glass-ceramics [22], and bioactive glass/polymer composites [23]. It is interesting to note that, as shown in Fig. 9a, apatite particles tended to align themselves in a line at the early stage of SBF-soaking.

4. Discussions

Oxidation of CPTi using hydrogen peroxide at low temperatures ($\leq$ 80 °C) yielded amorphous titania gel on the CPTi surface [5,24]. Tengvall et al. [24] suggested that such gel consisted of Ti$^{4+}$(OH)$_2$O$^{2-}$, Ti$^{4+}$(OH)$^-$_2O$_2^-$, Ti$^{4+}$(OH)$_x$ and TiO$_2$$\cdot$$n$H$_2$O. In the current investigation, the endothermic peak appearing in the DSC curve confirms the existence of water molecules that were incorporated in the titania gel. TF-XRD analysis indicates that a mainly amorphous titania gel was obtained after oxidizing CPTi with the H$_2$O$_2$ solution at 80 °C for 2, 8 and 72 h (Fig. 5a). Crystallization of the amorphous titania gel can be realized through a simple hot water aging treatment at a low temperature of 80 °C (Fig. 5b).

Matsuda et al. [25,26] reported the formation of anatase nanocrystals within a silica–titania gel (SiO$_2$ : TiO$_2$ molar ratio
of 5:1) containing poly(ethylene glycol) (PEG) through a hot water treatment (97 °C). They postulated that the anatase was a result of hydrolysis of the Si–O–Ti bonds. During hot water treatment, PEG was leached out and the resultant porous structure of the gel film accelerated hydrolysis and enhanced homogeneous nucleation of anatase nanocrystals throughout the film. As a support for such postulation, they noticed that the formation of anatase was scarcely observed in the sol–gel derived pure titania films that had been subjected to the same hot water treatment. Although the amorphous gel obtained in the current investigation was pure titania, it had a highly porous structure, which contributed to the low-temperature crystallization in a similar way to what the porous structure obtained by Matsuda et al. through PEG leaching had done. During hot water aging, hydrolysis of the Ti–O–Ti bonds was made possible by the highly porous structure, which increased the surface area of titania gel that was exposed to water. Anatase nanocrystals were then precipitated back onto the CPTi substrate from the saturated Ti(OH)4 solution. Morphological changes shown in Figs. 1–3 supported strongly such dissolution–precipitation mechanism. According to the passivity theory, titanium in contact with electrolytes has active and passive surfaces simultaneously and undergoes a continuous process of partial dissolution and reprecipitation [27]. Therefore, dissolution–precipitation of the titania film, which is favored by the porous structure as well as hot water, appears to be highly possible.

SBF-soaking experiments revealed that, even after immersion for one week, there was no apatite deposition on the

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**Fig. 5.** TF-XRD patterns of CPTi oxidized by H2O2 solution at 80 °C for various times (a), followed by hot water aging at 80 °C for 72 h (b).

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**Fig. 6.** DSC curve of titania powders detached from the CPTi surface oxidized by H2O2 solution at 80 °C for 72 h.

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**Fig. 7.** FT-IR spectra of CPTi oxidized by H2O2 solution at 80 °C for 8 h and aged in hot water at 80 °C for 72 h, followed by SBF-soaking for various times.
amorphous titania gel. However, 24 h were sufficient to induce the formation of an apatite layer on the low-temperature crystallized anatase film. The excellent apatite-inducing ability of CPTi having undergone hot water aging can be ascribed to the large amounts of Ti–OH groups preserved in the anatase film, which is only possible with the low-temperature, soft solution approach.

The SBF is a supersaturated calcium phosphate solution with respect to apatite. Therefore, the rate-controlling step for apatite deposition in SBF is its nucleation on various substrates [28]. On one hand, in the current investigation, water molecules attached to the amorphous titania gel, which desorbed only after heating above 180 °C (refer to the DSC result shown in Fig. 6), would remain in the anatase film after the hot water aging. They decreased the contacting angle between SBF and the substrates. Therefore, a reduced nucleation energy and hence an enhanced apatite nucleation rate can be expected. On the other hand, the abundant Ti–OH functional groups produced by H$_2$O$_2$ oxidation, which were not lost because no heat treatment was used in the current investigation, would attract calcium ions, which in turn would adsorb phosphate ions in SBF to initiate apatite deposition thus benefiting the formation of an apatite layer [12,29]. The large amount of Ti–OH groups on the substrate surface also favors preferential adsorption of phosphate ions through the reaction shown below:

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\text{Ti(OH)}_3^{3+} + \text{HPO}_4^{2-} \rightarrow \text{Ti}^{4+} \text{PO}_4^{3-} + \text{H}_2\text{O}
\]

where (ads), (ox) and (aq) denote adsorbed ions and ions in oxide and aqueous solution, respectively. Calcium phosphate therefore nucleates by the adsorption of calcium ions on the adsorbed phosphates.

The exclusion of thermal treatment from surface modification techniques also avoids possible damages in mechanical properties to CPTi implants that thermal treatment may cause. This, together with the excellent in vitro bioactivity achieved for CPTi, suggests that the present low-temperature surface modification approach is a viable way of providing Ti implants with desired bioactivity and for achieving an early and stable fixation of Ti implants.

5. Conclusions

Well-crystallized anatase thin films with excellent in vitro bioactivity could be produced on titanium surfaces by soaking the titanium substrate in 30 mass% H$_2$O$_2$ solution at 80 °C for 2 to 72 h, followed by a hot water aging at 80 °C for 72 h. During hot water aging, the amorphous titania gel produced by H$_2$O$_2$ oxidation hydrolyzed and re-precipitated back to the substrate to form anatase nanocrystals. Titanium treated by the present low-temperature chemical modification technique could induce apatite formation in the simulated body fluid within 24 h.

Acknowledgements

The authors would like to thank the technical staff in the different universities for their assistance. The work reported in this paper was supported in part by a research grant from the University of Hong Kong, and the Natural Science Foundation of Zhejiang Province (Project No. M503011).
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