TITANIA DERIVED FROM COMBINED CHEMICAL AND THERMAL TREATMENTS OF TITANIUM : *IN VITRO* APATITE FORMING ABILITY

Akiyoshi Osaka^{1,2*}, Kanji Tsuru^{1,2}, Satoshi Hayakawa¹

(*Corresponding author: osaka@cc.okayama-u.ac.jp)

¹Biomaterials Lab., Faculty of Engineering, Okayama University,
3-1-1 Tsushima, Okayama 700-8530 Japan

²Research Center for Biomedical Engineering, Okayama University,
3-1-1 Tsushima, Okayama 700-8530 Japan

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Abstract: Chemical treatment of a Ti substrate with 6 mass% H₂O₂ and subsequent thermal treatment yielded surface titania gel with enhanced ability of spontaneous *in vitro* deposition of apatite or bioactivity. We examined the effects of the chemical treatment time and the concentration and pH of the H₂O₂ solutions on both the formation of titania gel and *in vitro* bioactivity. The optimum treatments consisted of 6 mass% H₂O₂ treatment at 60°C for 3-6 h and subsequent thermal treatment at 400 °C for 1 h. The formation of the titania gel layer proceeded in a pH range of 4 to 4.6, while the corrosion of the titania gel layer was enhanced at a pH below 4.3. The reactions between the titanium substrates and H₂O₂ involved the oxidation of titanium, titania gel formation and Ti (IV) dissolution. The optimized chemical and thermal treatments (CHT method) were applicable for providing a Ti mesh screen with high *in vitro* apatite-forming ability as well as Ti plates with or without grooves.

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INTRODUCTION

Commercially available pure titanium (c.p. Ti) and its alloys are widely used for dental and orthopedic implants because of their high fracture toughness and good biocompatibility, although they cannot directly bond to bone. Thus, their fixation to human bone is mainly achieved by mechanical interlocking such as screwing and bone cementing, though such fixation sometimes has lead to loosening during long-term implantation. In order to overcome this problem, several chemical methods for surface modification of titanium implants have already been proposed to provide them with the ability of direct bonding to bone tissues. Note that essential for such tissue-implant bonding is the *in vivo* apatite-forming ability of implant materials.¹⁻⁴ Chemical treatments of titanium substrates proposed so far¹⁻⁸ are primarily aimed at controlling the formation of titania²⁻⁸ or sodium titanates¹ on the titanium substrate surfaces that induced apatite formation when those substrates were soaked in a simulated body fluid (SBF of the Kokubo's recipe⁹). The SBF was similar in inorganic ion composition to human blood plasma. Wang *et al.*^{5,6,8} improved the apatite-forming ability of titanium substrates treated with a hydrogen peroxide solution containing TaCl₅ by subsequent thermal treatments.

Recently, we confirmed that c.p. Ti mesh screens treated with a 30 mass% H₂O₂ solution (60°C, 24h) and subsequent thermal (400°C, 1h) treatment could

deposit a dense layer of apatite particles (~10µm in diameter) on the surface of each component wire in SBF within 3 days⁷. This implies that the combined chemical and thermal treatments (CHT method) can be applied for providing the c.p. Ti mesh screens with bioactivity and would be a new strategy for apatite coating on various forms of titanium substrates by the use of such solutions similar to the body fluid. Previous CHT methods⁵⁻⁸ required a high concentration of H₂O₂ (30 mass%) or a low pH condition due to the addition of HCl or TaCl₅, as well as the chemical treatment for as long as 24 h at 60°C. However, we recently found that the need for long-time chemical treatment and a high concentration of H₂O₂ was heavily correlated to not only the manufacturer of titanium products but also the method of cutting a sheet of Ti into pieces of substrates. For example, a wire-cutting electric discharge machine (EDM) was used for preparing the pieces of titanium substrates in previous studies⁵⁻⁸. The EDM cutting leads to the formation of a new oxide layer on the titanium substrates. Our previous studies^{4,8} indicated that in order to provide bioactivity, these pre-formed oxide layers had to be removed by chemical polishing for 3 min with a 1.0 mass% HF aqueous solution and subsequent treatment for 5 min in a mixed solution of 1 mass% HF and 3 mass% H₂O₂ before the chemical treatment with the H₂O₂ solutions. In this study, titanium substrates were cut from a sheet of c.p. Ti with scissors. They were then rinsed three times with ion-exchanged distilled water for 5 min in an

ultrasonic cleaner. The surface reactivity of these titanium substrates with the H₂O₂ solutions employed here was greater than that of the titanium substrates used in previous studies⁵⁻⁸. Thus, the present study has found no need for such pre-treatments to remove the surface oxide layer. Impurities such as oxygen not only increase the strength of c.p. Ti but also reduce corrosion resistance¹⁰. Moreover, the manufacturing processes or the pre-treatments of the titanium substrates lead to the formation of a new oxide layer with unpredictable reactivity. Thus, it is not strange that a combination of the chemical (30 mass% H₂O₂, 60°C, 24h) and thermal (400°C, 1h) treatments could not always be reproducible in providing bioactivity. Furthermore, the system Ti-H₂O₂ involves such species as Ti, titania gel, Ti(IV) dissolved in solutions, Ti(OH)₄, and other complexes¹¹⁻¹³. Some of the formation reactions or decomposition reactions leading to those species may involve protons or oxonium ions. Thus, it is an important issue to control the chemical properties and microstructure of the titania gel layers by optimizing the H₂O₂ chemical treatment conditions so that the CHT method can be applied to various forms of c.p. Ti implants for dental and orthopedic surgery. Furthermore, the present CHT method offers an environmental advantage such as avoidance of salt by-products.

Tengvall *et al.*¹¹⁻¹³ described earlier the direct formation of titania gel in the Ti-H₂O₂ system. They simultaneously observed the decomposition of H₂O₂ to water and oxygen and the formation of the Ti(IV)-H₂O₂ complex without yielding the free oxygen radical in the catalytic decomposition of the Ti-H₂O₂ system. Eqs. (1) and (2) describe the reactions¹¹. When excess H₂O₂ is present in the solution, Ti(IV) forms Ti peroxide:

The peroxide is decomposed catalytically at the Ti surface through

Tengvall *et al.*¹¹ suggested that the radical TiO₂^{•-} is also derived either from TiO₂²⁻ due to oxidation of such species as OH⁻, HO₂⁻, and H₂O₂ or from the addition of hydroperoxyl (HO₂[•]) or hydroperoxide ions (HO₂⁻) to Ti(OH)₄. It is also suggested that stable TiO₂²⁻-TiO₂^{•-} or Ti(IV)-TiO₂^{•-} complexes are formed at or near the Ti surface during the catalytic process. Moreover, they referred to the formation of both a Ti(IV)-H₂O₂ complex with yellowish tint and polymeric matrix of Ti(OH)₄(aq)^{12,13}. The former complex indicated the formation of "a very stable end product, a TiOOH(aq) adduct, to which the superoxide radical O₂^{•-} may be bound." Unfortunately, the exact composition, Ti oxidation reaction process, or polymerization process of the titania gel are not still clarified. However, the reactions between the titanium substrate and H₂O₂ should be explained by considering that the oxidation of titanium, titania gel layer formation and Ti(IV) dissolution occurred

competitively under a specific temperature and specific pH condition.

Our study was concerned with the *in vitro* apatite-forming ability of the titania gel layers derived by the CHT method. Taking the pieces of Ti plates as the substrates, we examined how such factors as the chemical treatment time, the concentration of the H₂O₂ solutions, and their pH values affected the corrosion reactions of titanium and the formation of the crystalline phase in the titania gel layers after the thermal treatment at 400°C for 1 h. We also examined how those factors affected their *in vitro* apatite-forming ability. In order to show the bright prospect of the biomedical application of the CHT method for titanium substrates, the optimized CHT method was applied to the c.p.Ti substrates with parallel grooves and Ti mesh screens.

MATERIALS AND METHODS

A sheet of c.p. Ti was cut with scissors into pieces of titanium substrates 10x10x0.1 mm in size. They were then rinsed three times with ion-exchanged distilled water for 5 min in an ultrasonic cleaner. The surface reactivity of these titanium substrates with an H₂O₂ solution was higher than that of titanium substrates used in previous studies⁵⁻⁸. Thus, the present study found no need for such pre-treatments required previously for the removal of the surface oxide layer. The titanium substrates were then treated with 20 ml of 1, 3, 4.5, 6 or 10 mass% H₂O₂ solutions up to 12 h at 60°C. Each solution was denoted as an Hn solution. Those substrates were then washed with distilled water for 5 min in an ultrasonic cleaner. Subsequently, all the substrates were heat-treated at 400°C for 1h in air in an electric furnace. Those samples were denoted as Hn/mh: m stands for the period (m hour) of the treatment with the relevant Hn solution. The concentration of Ti(IV) dissolved from the titanium substrates into the Hn solutions was measured by inductively coupled plasma emission spectroscopy. The concentration of H₂O₂ in the Hn solutions was monitored by redox titration using KMnO₄. The pH values of the Hn solutions at 60 °C were measured using a pH meter with an Ag/AgCl reference electrode.

After the chemical and thermal treatments (the CHT method), the apatite-forming ability of the titanium substrates was examined *in vitro* by soaking them for 3 days in 20 ml of SBF⁹ at 36.5°C and pH 7.4. Their surface structure was investigated by thin-film X-ray diffraction (TF-XRD: CuKα), Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). For comparison, the CHT method was applied to a commercially available pure Ti mesh screen made of pure Ti wires 0.3 mm in diameter and pieces of c.p. Ti plates grooved at 300μm-intervals and 300μm in depth and width. The pre-formed oxide layer of c.p. Ti mesh screen was removed by chemical polishing for 3 min with a 1.0 mass% HF aqueous solution and subsequent soaking

for 5 min in a mixed solution of 1 mass% HF and 3 mass% H_2O_2 before the chemical treatments with the Hn solutions.

RESULTS

Corrosion of Ti due to the Hn solutions, crystallization of titania, and apatite formation on the Hn substrates were examined. The treatment of several titanium substrates with the Hn solutions precipitated slightly yellowish gel particles on their surfaces, accompanied by yellowish coloration of the Hn solutions. Table 1 summarizes the appearance of the substrates and solutions after the chemical treatment. With the H6 and H10 solutions, the amount of yellowish colloid particles increased and the solutions were tinted deeply. The precipitations were thoroughly removed by washing with distilled water for 5 min in the ultrasonic cleaner before subsequent thermal treatment.

Fig. 1 shows the change in the concentration of Ti(IV) dissolved in the Hn solutions as a function of the chemical treatment time. The Ti(IV) concentration increased with the reaction time. The slope of each curve represents the rate of corrosion of the Ti substrates, which increased in the order of the H_2O_2 concentration: H1 solution < H3 solution < H4.5 solution < H6 solution < H10 solution.

Fig. 2 shows the decrease in the H_2O_2 concentration of the Hn solutions as a function of the chemical treatment time. H_2O_2 was consumed by corrosion of the Ti substrates or other related reactions like the complex formation stated above. In accordance with the increase in the Ti(IV) concentration in Fig. 1, the rate of consumption of H_2O_2 increased in the order of the H_2O_2 concentration: H1 solution < H3 solution < H4.5 solution < H6 solution < H10 solution.

Fig. 3 shows the change in pH of the Hn solutions at 60°C as a function of the chemical treatment time. They had initial pH values of about 4.5 except for the H1 solution (5.4 in pH) because commercially

available 30 mass% H_2O_2 solutions were kept acidic with a stabilizer such as H_3PO_4 (3ppm) to suppress the decomposition of H_2O_2 . Note that the pH quickly decreased within 30-60 min and became almost constant after 3-6 h. The time for the minimum values of pH in each Hn solution increased in the order: H10 solution < H6 solution < H4.5 solution < H3 solution < H1 solution. This order was the inverse of that found above in Figs. 1 and 2.

Figure 1 Concentration of Ti(IV) in Hn solutions as a function of chemical treatment time.

Figure 2 Concentration of H_2O_2 for Hn solutions as a function of chemical treatment time.

Figure 3 Change in pH of Hn solutions at 60°C as a function of chemical treatment time.

Table 1 Formation of yellowish colloid particles and coloration the H_2O_2 solutions

Solution	Treatment time			
	1h	3h	6h	12h
1 mass% H1	-	-	-	-
3 mass% H3	-	-	-	○
4.5 mass% H4.5	-	-	-	●
6 mass% H6	-	-	●	●
10 mass% H10	○	●	●	●

- : No yellowish colloid particles and no coloration of the Hn solutions
 ○ : Yellowish colloid particles and no coloration of the Hn solution
 ● : Yellowish colloid particles and yellow coloration of the Hn solution

Figs. 4a-e show the TF-XRD patterns for the H1, H3, H4.5, H6 and H10 substrates after heat-treating at 400°C for 1h and subsequently being soaked in the

Figure 4a TF-XRD patterns for H1 substrates after being heat-treated at 400°C for 1h (left) and subsequently being soaked in SBF for 3 days (right). ▲:anatase, ▼:rutile, ○:apatite, ■:Ti

Figure 4b TF-XRD patterns for H3 substrates after being heat-treated at 400°C for 1h (left) and subsequently being soaked in SBF for 3 days (right). ▲:anatase, ▼:rutile, ○:apatite, ■:Ti

Figure 4c TF-XRD patterns for H4.5 substrates after being heat-treated at 400°C for 1h (left) and subsequently being soaked in SBF for 3 days (right). ▲:anatase, ▼:rutile, ○:apatite, ■:Ti

Figure 4d TF-XRD patterns for H6 substrates after being heat-treated at 400°C for 1h (left) and subsequently being soaked in SBF for 3 days (right). ▲:anatase, ▼:rutile, ○:apatite, ■:Ti

Figure 4e TF-XRD patterns for H10 substrates after being heat-treated at 400°C for 1h (left) and subsequently being soaked in SBF for 3 days (right). ▲:anatase, ▼:rutile, ○:apatite, ■:Ti

SBF for 3 days. Titanium diffractions (JCPDS 5-682) were detected for all substrates after heat-treating at 400°C for 1h. The appearance of crystalline titania phases depended on the treatment solutions and treatment time. No H1 substrates showed diffractions of anatase at ~25° in 2θ (JCPDS 21-1272) and rutile at ~26° in 2θ (JCPDF 21-1276) before they were chemically treated over 6 h. This indicated that chemical treatment longer than 6 h with the H1 solution was required to yield crystalline titania, i.e., anatase and rutile. The H3/6h substrate yielded anatase but no rutile. The anatase diffraction peak of the H3 substrates grew with the chemical treatment time. Anatase detected on the H4.5/3h and H6/3h substrates disappeared after 12 h of treatment. Rutile did not appear on the H3, H4.5 and H6 substrates. Strangely, no crystalline phases appeared on the H10 substrates by treatment of up to 12 h. The TF-XRD patterns for the H1, H3, H4.5, H6 and H10 substrates after soaking in SBF for 3 h indicated diffractions at 26° and 32° in 2θ due to apatite. H3/6h, H3/12h, H4.5/6h, H4.5/12h, H6/3h and H6/6h

deposited apatite whereas H1 and H10 did not. The XRD peak intensity at 26° and 32° indicated that H3/12h deposited more apatite than H3/6h, and that H6/6h deposited more than H6/3h. Note that apatite diffractions were not detected for the H6/1h, H6/12h, and H10 substrates. The XRD patterns in Figs. 4a-e indicated that the titanium substrates with stronger anatase diffractions gave stronger apatite diffractions after soaking in SBF for 3 days. Fig. 5 shows SEM images of the H1/6h and H6/6h substrates after soaking in SBF for 3 days. No change in surface morphology of the H1/6h substrate was observed after soaking in SBF for 3 days. In contrast, semi-spherical apatite agglomerates were observed on the H6/6h substrate within 3 days in SBF.

Figure 5 SEM images of H1/6h and H6/6h substrates after soaking in SBF for 3 days.

The effects of Hn solution pH on reactions with Ti substrates

In order to examine the effect of pH both on the corrosion reaction of titanium and on the crystallization of the titania gel layers due to the thermal treatment, the titanium substrates were treated with the H6 solutions whose initial pH values were adjusted at 3.5 and 5.1 with a dilute solution of HCl or NH_4OH , respectively. The substrates treated with the H6 solutions with HCl or NH_4OH for m hours were denoted as H6-HCl/mh or H6- NH_4OH /mh

substrates. Table 2 summarizes the appearance of the H6-HCl and H6- NH_4OH solutions as well as that of the H6 substrates after the H6-HCl/mh or H6- NH_4OH /mh treatments. Regardless of the initial pH values (3.5 or 4.5), the amount of yellowish colloid particles increased and the solutions were tinted deeply with the reaction time. On the other hand, no yellowish colloid particles were precipitated on the H6- NH_4OH substrates. Thus, the initial pH of the H6 solution, adjusted to 5.1, was effective in suppressing the precipitation of the yellowish colloid particles.

Fig. 6 shows the increase in the concentration of Ti(IV) in the H6, H6-HCl, and H6- NH_4OH solutions as a function of the chemical treatment time, indicating that some of the titania was dissolved into the solutions. The rate of dissolution increased in the order: H6- NH_4OH solution (pH: 5.1) < H6 solution (4.5) < H6-HCl solution (3.5) where the numbers in parentheses denote the initial pH values. Thus, corrosion was more rigorous in the solutions of lower pH. Fig. 7 shows the decrease in the H_2O_2 concentration of the solutions of the H6 family as a function of chemical treatment time. The rate of H_2O_2 consumption increased in the order: H6 solution < H6- NH_4OH solution < H6-HCl solution. The order of the rate of consumption disagreed with that for increasing Ti(IV) indicated in Fig. 6. Thus, some

Table 2 Formation of yellowish colloid particles and coloration of the H_2O_2 solutions of family

Solution	Treatment time			
	1h	3h	6h	12h
6 mass% H6	—	—	●	●
6 mass% HCl H6-HCl	—	▲	●	●
6 mass% NH_4OH H6- NH_4OH	—	—	—	▲

— : No yellowish colloid particles and no coloration of the H6 solutions
 ▲ : No yellowish colloid particles but yellow coloration of the H6 solutions
 ● : Yellowish colloid particles and yellow coloration of the H6 solutions

Figure 6 Concentration of Ti(IV) in H6-HCl, H6 and H6- NH_4OH solutions different in initial value of pH.

of the H_2O_2 molecules were consumed in dissolution of Ti(IV) into the H6 solutions and the others were involved in the formation of colloid and oxide layers. Moreover, since the differences in the rate of H_2O_2 decomposition among the solutions were small but extinct, the HCl and NH_4OH additives catalyzed the reactions between the Ti substrates and H_2O_2 , though slightly. Fig. 8 shows the change in the pH of the H6 solutions as a function of chemical treatment time. Note that the pH quickly decreased within 30-60 min and became almost constant after 1 h except for the H6 solutions. As described later in eq. (3) after Tengvall *et al.*¹¹⁻¹³ or Sugimoto *et al.*¹⁴, the oxidation reactions of Ti with H_2O_2 yield hydronium ions or protons, hence the pH of the H_2O_2 solution increases. Thus, a minimum in pH means that the other reactions that consume the protons become more rigorous than the oxidation of Ti. In this respect, the minimum in pH has some importance. The minimum values of pH in the H6 solutions were larger in the order: H6-HCl solution < H6 solution < H6- NH_4OH solution. This order was just the inverse order found for the Ti(IV) concentration. Thus, the dissolution of Ti(IV) was phenomenally in direct relation with the

value of lowest pH in each H6 solution. In addition, Fig. 8 shows that the time until the minimum pH values were reached was longer in the order: H6-HCl < H6- NH_4OH solution < H6 solution

Fig. 9 shows the TF-XRD patterns for H6 substrates treated with the H6-HCl and H6- NH_4OH solutions before being heat-treated at 400°C for 1h. The titanium diffractions were detected for all the substrates after being heat-treated at 400°C for 1h. H6-HCl /3h showed the diffractions of anatase and rutile, while the H6-HCl substrates chemically treated over 6h showed no deposition of any crystalline phases. The latter result indicates that the least amount of titania gel formed. The anatase diffraction was also detected for the H6- NH_4OH substrates chemically treated over 3 h and it grew with treatment time of up to 12h. In contrast, Fig. 4(d) indicated that anatase disappeared from the H6 substrates after 12 h of treatment. Thus, the titania gel formation was favored by the H6- NH_4OH solution having an initial pH of 5.1, while it was suppressed by the H6-HCl solution having an initial pH of 3.5. From both the effects of pH exhibited in Figs. 3 and 8 and the change in the intensity of anatase diffractions in Figs.4a-e and 9, one can elucidate that the formation of the titania gel layer proceeded in the pH range of 4 to 4.6. The results indicated in Figs. 4, 6, and 9 confirm that deposition and dissolution of titania could take place simultaneously. Also, Figs. 1, 3 and 6 indicated that the concentration of Ti(IV) dissolved in the range of pH < 4.3 was much greater than that between pH 4.3 and 4.6. This means that the dissolution of the titania gel layer was enhanced in the range of pH < 4.3. From the results above, it is indicated that in order to yield a titania gel layer the pH values of the H_2O_2 solutions should be kept above 4 within 12h when chemically treating the Ti substrates; otherwise the titania gel layer would be dissolved or disappear during the chemical treatment.

Figure 7 H_2O_2 concentration of H6-HCl, H6 and H6- NH_4OH solutions different in the initial value of pH.

Figure 8 Change in pH of H6-HCl, H6 and H6- NH_4OH solutions as a function of chemical treatment time.

Figure 9 TF-XRD patterns for H6 substrates treated in H6 solutions with different pH after being heat-treated at 400°C for 1h. ▲: anatase, ▼: rutile, ■: Ti

Surface morphology of a Ti mesh and grooved substrates

Figs. 10a) and b) show the SEM images of the Ti mesh screens subjected to the H6/3h treatment; soaking in the H6 solution at 60°C for 3h and subsequently heating at 400°C for 1h. A number of etch pits of ~10 µm in size are indicated. Figs. 10c) and d) exhibit the surface microstructure of those meshes after soaking in SBF for 3 days. Apatite particles (~10µm in diameter) covered the whole surface of the component wires. Fig. 11 shows a SEM image of the grooved c.p. Ti plate subjected to the H6/6h treatment and subsequently soaked in SBF for 3 days. Apatite particles of ~10µm in diameter densely covered the whole surface. These results indicated that the present CHT method is a powerful technique for bone-like apatite coating on various forms of titanium substrates by the use of SBF.

DISCUSSION

Titania Gel Layer Formation on Titanium Substrates

Since anatase was a product of crystallization of amorphous titania or titania gel, the XRD intensity of anatase in Figs. 4 and 9 implied that the amount of titania gel increased in the order: in H6-HCl solution < H1 solution < H3 solution < H4.5 solution < H6 solution ≤ H6-NH₄OH solution. That is, the H6 and H6-NH₄OH solutions yielded the greatest amount of titania gel. Thus, the results strongly suggest that more concentrated H₂O₂ solution gave more titania gel, hence, it might favor the precipitation of crystalline titania. However, anatase that appeared on the H4.5/3h and H6/3h substrates reduced the diffraction peak intensity due to the H4.5/12h or H6/12h treatment. The H6-HCl/6h and H6-HCl/12h substrates did not deposited any crystalline phases. None of the H10 family precipitated any crystalline titania phases but rather yellowish gel particles. These results cannot be explained only on the basis of the H₂O₂ concentrations as long as the titania precipitation and dissolution of Ti(IV) were dependent on the pH of the treating solutions. Thus, it is necessary to take into account the effect of the pH of these Hn solutions on the formation of the titania gel layer.

Tengvall *et al.*¹¹⁻¹³ described earlier the direct formation of titania gel in the Ti-H₂O₂ system, as stated above. Although the initial values of pH of the H₂O₂ solution in the present study ranged from 3.5 to 5 and were higher than that of Tengvall and his coworkers (pH < 3)¹¹, similar phenomena were observed above, such as the decrease in pH in the first reaction stage, consumption of H₂O₂, and formation of a yellowish Ti(IV)-H₂O₂ complex. Among several species involved in the Ti-H₂O₂ system, Ti(OH)₄(aq) was stable only under highly acidic conditions (pH < 3) after Mühlebach *et al.*¹⁵ Also, they reported¹⁵ that in the Ti(IV)-H₂O₂ system at a pH higher than 3 at a low temperature (-30°C),

Figure 10 SEM images of a dense apatite layer covering the c.p. Ti mesh subject to the H6/6h treatment before and after being soaked in SBF for 3 days.

Figure 11 SEM images of a dense apatite layer on the c.p. Ti plate having grooves at 300-μm intervals, and 300 μm in width and depth, subject to H6/6h treatment after being soaked in SBF for 3 days.

- the condensing species were anions, $\text{Ti}_2\text{O}_5(\text{OH})_x^{(x-2)-}$ ($x \geq 2$) or $\text{Ti}_2\text{O}_5(\text{OH})_2$,
- the precursors of the final precipitate must also be negatively charged polymers, and the final precipitate is peroxotitanium hydrate $\text{TiO}_3(\text{aq})$, consisting of Ti_2O_5 units,
- the anion condensation was accompanied by an increase in the pH, hence it was much faster with increasing pH.

Indeed, our previous FT-IR studies⁶⁻⁹ confirmed the presence of -O-O- groups of Ti_2O_5 units on the titanium surface treated with an H_2O_2 solution; an IR absorption peak assignable to the -O-O- groups was observed at 890 cm^{-1} . Since the temperature of the present chemical treatment, 60°C , was much higher than that in the experiment by Mühlebach et al.¹⁵, it is suggested that a fraction of the Ti-O-O-Ti links of the Ti_2O_5 units is cleaved to the Ti-O-Ti links yielding $\text{TiO}_2(\text{aq})$. Therefore, one can expect that the Ti(IV)- H_2O_2 complexes that formed under the present chemical treatment conditions involve $\text{TiOOH}(\text{aq})$, $\text{TiO}_3(\text{aq})$ and $\text{TiO}_2(\text{aq})$.

In order to discuss both the formation of $\text{TiOOH}(\text{aq})$ and $\text{TiO}_2(\text{aq})$ (hydrated titania gel) and the dissolution of $\text{TiO}_2(\text{aq})$, a series of reactions leading to direct formation of titanium peroxides in

the titanium and hydrogen peroxide system ($\text{Ti}-\text{H}_2\text{O}_2$) were considered on the basis of Tengvall *et al.*^{5,6}. They involve some redox reactions that the derivative species are subsequently subject to following.

Here, eq. (3) shows that TiOOH is derived from an H_2O_2 solution accompanying the formation of protons or oxonium ions. $\text{TiOOH}(\text{aq})$ may stay either on the substrate surface or in the solution. In eq. (4) the hydrated neutral complex $\text{Ti(OH)}_4(\text{aq})$ is derived from $\text{TiOOH}(\text{aq})$, consuming the oxonium ions, while eq. (5) describes the formation of hydrated titania gel, $\text{TiO}_2(\text{aq})$, from $\text{Ti(OH)}_4(\text{aq})$. Eq. (6) shows that Ti(OH)_4 changes to the positively charged complex Ti(OH)_{4-n}^{n+} , after Sugimoto *et al.*¹⁵. In eq. (7) dissolution of the hydrated titania gel proceeds at the expense of protons or oxonium ions. Thus, the pH decreases in eq. (3) while it increases in eqs. (4), (6), and (7). Therefore, the decrease in the pH suggests either the release of TiOOH into the H_2O_2 solutions or the formation of $\text{TiOOH}(\text{aq})$ on the Ti substrates. The increase in the pH suggests, on the other hand, the formation of Ti(OH)_4 and Ti(OH)_{4-n}^{n+} , as well as the dissolution of $\text{TiO}_2(\text{aq})$. However, the system may involve some important chemical species, including the $\text{TiO}_3(\text{aq})$, superoxide radical O_2^- , stable complex $\text{TiO}_2^{2-}-\text{TiO}_2^{2-}$, OH^- , hydroperoxyl (HO_2), the hydroperoxide ion (HO_2^-) or TiO_2^{2-} that have not been considered in eqs. (3) through (7). Nevertheless, eqs. (3)-(7) will be used in the following discussion because they well describe the correlation among the possible reactions and changes in the pH.

According to eq. (3), the concentration of Ti(IV) dissolved in the Hn solution, C_{Ti} , is a function of the pH of the solutions as far as a sufficient amount of Ti is present in the system. Therefore, knowing C_{Ti} and the initial pH value of the solution, pH_i , one can predict the pH of the treatment solution with eq. (8):

$$\text{pH} = \text{pH}_i - \log(C_{\text{Ti}}) \quad (8)$$

Fig. 12 compares the experimental and calculated values of the pH for the H6- NH_4OH , H6, and H6-HCl solutions as a function of the treatment time. Note that the calculated values of pH are lower than the empirical values of pH. This means that protons or oxonium ions have been consumed in the course of the Ti- H_2O_2 reactions, hence it is necessary to take the H^+ consumption in eqs. (4) and (6) into account to describe the system. Then, from those equations, the concentration of the consumed H^+ , DH^+ is a function of the empirical pH_{ex} , and calculated pH_c :

$$\log \text{DH}^+ = -\text{pH}_c / \text{pH}_{\text{ex}} \quad (9)$$

Fig. 13 plots DH^+ for those H6 solutions on the basis of eq. (9) as a function of the treatment time. Regardless of the solution, DH^+ increased in the order: H6- NH_4OH solution < H6 solution < H6-HCl solution. The dissolution reactions are in equilibrium with the inverse reactions, and a larger concentration of protons favors the dissolution of titania.

Accordingly, it is reasonable that this order agreed with that found in Fig. 6 for the concentration of Ti(IV) during the treatment time. The $\square H^+$ versus time curve for the H6 solution (4.5 in pH) shows a sudden increase in the slope at 6 h since then the XRD intensity of anatase decreased in Fig. 4d. Also, it is noted that

- in the first 6 hours the values of $\square H^+$ and the slope of the curve for the H6 solution well agreed with those for the H6-NH₄OH solution.
- in the period longer than 6 h $\square H^+$ for the H6 solution increased parallel to that of the H6-HCl solution.

Therefore, it is empirically confirmed that the dissolution of the crystalline titania or titania gel accompanies the consumption of protons: as far as

Figure 12 Comparison between experimental and calculated value of pH in H6 solutions at 60°C.

Figure 13 Change in consumed H^+ concentration of H6 solutions with different pH as a function of chemical treatment time.

equations (3) through (7) above are concerned, the formation of $Ti(OH)_4$ and $Ti(OH)_{4-n}^{n+}$ is involved. Thus, the similarity in the change in $\square H^+$, found among three H6 solutions with different pH, should be correlated to similar rates of the formation or dissolution of the titania gel layer, $TiO_2(aq)$, between the two solutions. The gentle slope for the H6 and H6-NH₄OH solutions in the range of < 6h in Fig. 13 suggests the formation of a titania gel layer on the titanium substrates. Already observed were the slight increase in pH for the H6 treatment (>6h) in Fig. 3, the decrease in the XRD intensity of anatase in Fig. 4d, and the increase in $\square H^+$ for the H6 solution after 6 h in Fig. 13. From those results it is derived that the titania gel was dissolved at a pH below 4. One can propose two possible mechanisms for the reduction or disappearance of the titania gel layer:

- The gel is modified to the charged complex, $Ti(OH)_{4-n}^{n+}$ due to eq.(6) and promotes the formation of yellowish colloid particles at a pH below 4, while the neutral complex $Ti(OH)_4$ in the solution is deposited on the titanium substrates resulting in the titania gel (eq. (5)). Those two are equilibrated.
- The stability of the titania gel $TiO_2(aq)$ is reduced with a decrease in the pH of the H_2O_2 solutions, and the gel is dissolved into Ti(IV) at the expense of H^+ (eq. (7)). This process is also in equilibrium with respect to pH when Ti(IV) is not saturated.

Thus, the reaction between the titanium substrate and H_2O_2 can be interpreted by considering that the oxidation of titanium, titania gel layer formation and Ti(IV) dissolution occurred competitively under a specific temperature and specific pH condition. Fig. 14 illustrates the mechanism of the titania gel layer formation on the titanium substrates in the H_2O_2 solutions.

Yellowish Titania Colloid Particles

We already touched on the yellowish titania colloid particles that formed and easily adhered to the surface of the titanium substrates. They were thoroughly removed by washing with distilled water in an ultrasonic cleaner or were easily peeled off after drying. Tables 1 and 2 indicated that these yellowish titania particles tended to form at high concentrations of H_2O_2 and at a low pH. Indeed, the H6-HCl solution and H10 solution yielded the yellowish titania colloid particles formed outside and adhered to the surface of titanium substrates, but did not yield the titania gel layer. Furthermore, naked-eye inspection indicated that the titania colloid particles were distributed heterogeneously on the titanium substrate surface. Such Ti substrates with a heterogeneous and fragile titania colloid particle layer are not applicable for dental and orthopedic surgery because a strong and stable fixation at the interface between tissue and implant is required. Recently, Wu *et al.* reported the low-temperature (80°C) process of preparing crystalline titania (anatase and rutile) through interactions between c.p. Ti and hydrogen peroxide solutions¹⁶⁻¹⁹ based on the chemical treatment condition of Wang *et al.*^{5,6}. Since the process of Wu

Figure 14 Mechanism of titania gel layer formation on titanium substrate in H₂O₂ solution.

*et al.*¹⁶⁻¹⁹ does not include the thermal treatment at 400°C for 1h, many TiOH groups may remain in the crystalline titania gel. Both Wang *et al.*^{5,6} and Wu *et al.*¹⁶⁻¹⁹ reported that thus-obtained crystalline titania had a high *in vitro* apatite-forming ability within 1 day. However, their chemical treatment required a high concentration of H₂O₂ (15-30 mass%) or a low pH condition (HCl or TaCl₅ additive) in order to get enough precipitates of titania colloid particles to coat the surface of titanium substrates homogeneously and obtain a crystalline titania gel layer. However, most of the layer consisted of precipitates or weakly adhering titania colloid particles. Such fragile titania colloid particle layers formed at a high H₂O₂ concentration and a low pH condition are also not applicable as such for dental and orthopedic surgery and hence their mechanical and adhesion strength should be improved in future.

in vitro Apatite-forming Ability of Titania Gel Layer

After Li *et al.*^{20, 21} the titania gel prepared by the sol-gel process induced apatite formation on its surface when soaked in SBF for 2 weeks. The Ti-OH groups in the titania gel were suggested to favor the deposition of calcium phosphates by providing negatively charged sites, hence being the targets of adsorbing calcium and phosphate ions to form apatite nuclei. The present chemical treatment with H₂O₂ could yield a titania gel involving TiOOH(aq), TiO₃(aq), and TiO₂(aq) on the titanium substrates. Therefore, the chemical bonding states around Ti atoms in these derived titania gels are very different from those in the sol-gel derived titania gels. For example, titanium substrates treated with an H₂O₂ solution developed thin titania layers, which unfortunately could not deposit apatite⁷ unless they were heat-treated⁵⁻⁸ or were chemically treated with the low-temperature process for crystalline titania proposed by Wu *et al.*¹⁶⁻¹⁹ Wälivaara *et al.*¹³ reported that the chemical treatment in H₂O₂ enhanced calcium adsorption onto Ti surfaces. One could

consider at least two types of negatively charged sites in the titania gels formed on titanium substrates. One is the TiOH group, and the other is the TiOOH group. Wang *et al.*⁶ suggested that thermal treatment at 400°C eliminated the Ti-O-O-Ti and TiOOH groups from the titania gel derived by H₂O₂ treatment and promoted the formation of TiO₂(anatase), resulting in enhanced apatite deposition. Such elimination of the -O-O- groups was confirmed by FT-IR spectroscopy (not shown here). The IR absorption peak at 890 cm⁻¹ assignable to -O-O- groups disappeared due to thermal treatment at 400°C for 1h. Moreover, Figs. 4a and 4e above showed that the H1 and H10 substrates precipitated no anatase in spite of thermal treatment at 400 °C for 1h and could not deposit apatite after soaking in SBF for 3 days. The XRD patterns in Figs. 4a-e already indicated the correlation of the titanium substrate having more anatase to the deposition of more apatite. That is, it was also confirmed that the titanium substrates with titania gels consisting of a lot of anatase favored the deposition of apatite, as pointed out by Wang *et al.*⁶. Contradictory results were also reported. Hayakawa *et al.*⁷ recently treated Ti chemically with H₂O₂ containing TaCl₅ and subsequently heated at 400°C for 1h. They reported that the combined treatment yielded titania gels involving anatase and rutile together with an amorphous phase, that those gels also favored the deposition of apatite and that their apatite-forming ability was even better than gels with anatase only⁷. They supposed⁷ that the Ta(V) additive suppressed not only the formation of anatase but also the decrease in the Ti-OH groups due to the elimination of H₂O, and that subsequent thermal treatment led to sintering and polymerizing the Ti-O bonds and to crystallization of anatase or rutile. The elimination of the -O-O- groups has been confirmed above to proceed during the thermal treatment. Furthermore, a few research groups reported that the Ta(V) species with TaOH groups favored the deposition of apatite²²⁻²⁶. However, the *in vitro* apatite-forming ability of the Ta(V) species is not so high as to deposit apatite within 3 days in SBF. For example, Miyazaki *et al.* reported that the TaOH groups on a tantalum metal substrate required at least 1 week to induce apatite nucleation in SBF²⁵. Thus, the primary factors for favoring good *in vitro* apatite-forming ability of the titania gel derived by chemical treatment with H₂O₂ containing TaCl₅ and subsequent thermal treatment should be the elimination of the -O-O- groups, the ratio of TiOOH to TiOH, the amount of active negatively charged sites, and the favorable structure of the gel itself. One of the subdominant factors is the Ta(V) species.

Kim *et al.*¹ reported that the treatment of titanium substrates with an aqueous solution of NaOH, followed by heat treatment at 500-800°C, results in a thin sodium titanate layer, which then formed a dense bone-like apatite layer when soaked in SBF. Recently, Takadama *et al.*²⁷ reported that the sodium titanate layer yielded Ti-OH groups due to ion-exchange between the sodium ions in the layer

Osaka A, Tsuru K, Hayakawa S

and oxonium ions (H_3O^+) in SBF, and that these Ti-OH groups induced apatite nucleation. Note that the bioactivity of the Ti-OH groups derived from those sodium titanate layers depended heavily on both increase in the pH near the gel-SBF interface due to sodium ion release from the gel and on thus-induced further increase in the degree of supersaturation of the SBF for apatite. On the other hand, the titania gel layers derived from the present CHT method would not induce any extra increase in the degree of supersaturation of the SBF for apatite because they could not increase in either pH or Ca(II) and P(V) concentrations. Still, the present gel layers could deposit dense layers of apatite particles in SBF within 3 days. Their *in vitro* bioactivity was much greater than the sol-gel derived titania gel that required soaking in SBF for 2 weeks before deposition of apatite.^{20,21}

Surface Microstructure

Several research groups have examined the effects of the pore structure or the textural features of sol-gel derived silica gel on apatite formation²⁹⁻³³. Pereira et al. suggested that the critical pore size for rapid apatite formation is larger than 2 nm²⁹, while Peltola et al. suggested that a great mesopore volume and a wide distribution of mesopores (2-50 nm) favors apatite nucleation on the pure silica³². Peltola et al.³⁴⁻³⁶ also reported that the pore structure and thickness of sol-gel derived titania film do not have a clear effect on apatite formation. They suggested³⁶ that

- the structure of the outermost surface in nanometer scale is of importance for bioactivity,
- the surface topographical peak distance distribution between 15-50 nm favors apatite nucleation, and
- peak distances greater than 50 nm do not have any significant effect on *in vitro* bioactivity.

Fig. 15 shows a SEM image of H6/6h substrates before soaking in SBF, indicating that a number of submicrometer-scale pores (100-300 nm) formed by the CHT method. Recently, Wang et al.⁸ and Wu et al.¹⁹ reported similar submicrometer-scale pores formed on the titania gel layer 0.3-0.6 μm in thickness. However, the relation between the morphology of the titania gel layer derived by the CHT method and apatite-forming ability remains unclear in this study. The effect of the morphology of titania gel on the apatite-forming ability and its surface charge density is an issue for future work.

In conclusion, we can emphasize that the high apatite-forming ability of the titania gel originates from the favorable structure of the gel itself. On the basis of the structural matching rule between TiO_2 (anatase) and $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ (hydroxyapatite) as proposed by Wang et al.⁷ or Mao et al.²⁸, anatase favored the deposition of apatite. Therefore, one can conclude that the present CHT method is convenient for providing pieces of both Ti and Ti mesh screen with high *in vitro* apatite-forming ability. The CHT method could be applied to various forms of c.p. Ti implants for dental and orthopedic surgery. Furthermore, we expect that the CHT method can

Figure 15 SEM image of nm-sized porous surface of H6/6h substrate.

provide a high blood-compatible titania gel^{37,38} which is promising for artificial heart valves and coronary stent made of c.p. Ti.

CONCLUSIONS

We could provide titanium substrates with *in vitro* apatite-forming ability by means of 6 mass% H_2O_2 treatment, a more dilute one than that previously employed by Wang et al.^{5,6,8}, at 60 °C for 3-6 h and subsequent thermal treatment at 400 °C for 1 h. The reactions between the titanium substrate and H_2O_2 were interpreted by considering that the oxidation of titanium, titania gel formation and Ti(IV) dissolution occurred competitively. The rates of titania gel formation in 1 and 3 mass% H_2O_2 treatments were smaller than that in 6 mass% H_2O_2 treatment, while the Ti(IV) dissolution from titania gel occurred in 10 mass% H_2O_2 treatment more preferentially than in 6 mass% H_2O_2 treatment. The titania gel formation was controlled not only by the H_2O_2 concentration but also by the pH. The formation of the titania gel layer proceeded in a pH range of 4 to 4.6, while the corrosion of the titania gel layer was escalated at a pH below 4.3. The optimized combined chemical and thermal treatments (CHT method) is applicable for providing both Ti plates with grooves and Ti mesh screens with high *in vitro* apatite-forming ability.

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