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.

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. Chemical treatments of titanium substrates proposed so far are primarily aimed at controlling the formation of titania on the titanium substrate surfaces that induced apatite formation when those substrates were soaked in a simulated body fluid (SBF of the Kokubo's recipe). The SBF was similar in inorganic ion composition to human blood plasma. Wang et al. 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 days. 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 methods 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 studies. The EDM cutting leads to the formation of a new oxide layer on the titanium substrates. Our previous studies 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 those titanium substrates with the H$_2$O$_2$ solutions employed here was greater than that of the titanium substrates used in previous studies$^{5-8}$. 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 resistance$^{9}$. 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% H$_2$O$_2$, 60°C, 24h) and thermal (400°C, 1h) treatments could not always be reproducible in providing bioactivity. Furthermore, the system Ti-H$_2$O$_2$ involves such species as Ti, titania gel, Ti(IV) dissolved in solutions, Ti(OH)$_4$, and other complexes$^{11-13}$. 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 H$_2$O$_2$ 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-H$_2$O$_2$ system. They simultaneously observed the decomposition of H$_2$O$_2$ to water and oxygen and the formation of the Ti(IV)-H$_2$O$_2$ complex without yielding the free oxygen radical in the catalytical decomposition of the Ti-H$_2$O$_2$ system. Eqs. (1) and (2) describe the reactions$^1$. When excess H$_2$O$_2$ is present in the solution, Ti(IV) forms Ti peroxide:

$$\text{Ti(IV)} + \text{H}_2\text{O}_2 \rightarrow \text{TiO}_2^{2-} + 2\text{H}^+ \quad (1)$$

The peroxide is decomposed catalytically at the Ti surface through

$$\text{TiO}_2^{2-} + \text{H}^+ \rightarrow \text{Ti(IV)} + \text{HO}_2^- \quad (2)$$

Tengvall et al.$^{11}$ suggested that the radical TiO$_2^-$ is also derived either from TiO$_2^{2-}$ due to oxidation of such species as OH$,^+, \text{HO}_2$, and H$_2$O$_2$ or from the addition of hydroperoxyl (HO$_2$) or hydroperoxide ions (HO$_2^-$) to Ti(OH)$_4$. It is also suggested that stable TiO$_2^{2-}$-TiO$_2^-$ or Ti(IV)-TiO$_2^-$ complexes are formed at or near the Ti surface during the catalytic process. Moreover, they referred to the formation of both a Ti(IV)-H$_2$O$_2$ 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 O$_2^-$ may be bound." Unfortunately, the exact composition, Ti oxidation reaction process, or polymerization under the titania gel layer are not still clarified. However, the reactions between the titanium substrate and H$_2$O$_2$ 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 H$_2$O$_2$ 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 those titanium substrates with an H$_2$O$_2$ solution was higher than that of titanium substrates used in previous studies$^{5-8}$. 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% H$_2$O$_2$ solutions up to 12 h at 60°C. Each solution was denoted as an H$_n$ 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 1 h 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 H$_n$ 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 H$_2$O$_2$ in the Hn solutions was monitored by redox titration using K$_2$MnO$_4$. 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 SBF$^9$ 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
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for 5 min in a mixed solution of 1 mass% HF and 3 mass% H$_2$O$_2$ 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$_2$O$_2$ concentration: H1 solution < H3 solution < H4.5 solution < H6 solution < H10 solution.

Fig. 2 shows the decrease in the H$_2$O$_2$ concentration of the Hn solutions as a function of the chemical treatment time. H$_2$O$_2$ 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$_2$O$_2$ increased in the order of the H$_2$O$_2$ concentration: H1 solution < H3 solution < H4.5 solution < H6 solution < H10 solution.

Table 1 summarizes the appearance of the substrates and solutions after the chemical treatment. Table 1 summarizes the appearance of the substrates and solutions after the chemical treatment. Table 1 summarizes the appearance of the substrates and solutions after the chemical treatment.

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Table 1: Formation of yellowish colloid particles and coloration of the Hn solutions.

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.
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 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. 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 H3 substrates after being heat-treated at 400°C for 1h (left) and subsequently being soaked in SBF for 3 days (right). A:anatase, V:rutile, O:anatite, ■:Ti.
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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 H6/3h 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.

![H1 Substrate](image1.png)

![H6 Substrate](image2.png)

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 NH₄OH, respectively. The substrates treated with the H6 solutions with HCl or NH₄OH for m hours were denoted as H6-HCl/mh or H6-NH₄OH/mh substrates. Table 2 summarizes the appearance of the H6-HCl and H6-NH₄OH solutions as well as that of the H6 substrates after the H6-HCl/mh or H6-NH₄OH/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-NH₄OH substrates. Thus, the initial pH of the H6 solution, adjusted to 5.1, was effective in suppressing the precipitation of the yellowish colloid particles.

![Figure 6](image3.png)

Figure 6 Concentration of Ti(IV) in H6-HCl, H6-HCl, and H6-NH₄OH 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-NH₄OH 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 H₂O₂ concentration of the solutions of the H6 family as a function of chemical treatment time. The rate of H₂O₂ consumption increased in the order: H6 solution < H6-NH₄OH solution < H6-HCl solution. The order of the rate of consumption disagreed with that for increasing Ti(IV) indicated in Fig. 6. Thus, some
of the H$_2$O$_2$ 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$_2$O$_2$ decomposition among the solutions were small but extinct, the HCl and NH$_4$OH additives catalyzed the reactions between the Ti substrates and H$_2$O$_2$, 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.\textsuperscript{11-13} or Sugimoto et al.\textsuperscript{14}, the oxidation reactions of Ti with H$_2$O$_2$ yield hydronium ions or protons, hence the pH of the H$_2$O$_2$ 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$_4$OH 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$_4$OH solution < H6 solution.

Fig. 9 shows the TF-XRD patterns for H6 substrates treated with the H6-HCl and H6-NH$_4$OH 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$_4$OH 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$_4$OH 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$_2$O$_2$ 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](image1.png)

**Figure 7** H$_2$O$_2$ concentration of H6-HCl, H6 and H6-NH$_4$OH solutions different in the initial value of pH.

![Figure 8](image2.png)

**Figure 8** Change in pH of H6-HCl, H6 and H6-NH$_4$OH solutions as a function of chemical treatment time.

![Figure 9](image3.png)

**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),
Titania Derived From Combined Chemical and Thermal Treatments Of Titanium: In Vitro Apatite Forming Ability

The condensing species were anions, Ti$_2$O$_5$(OH)$_x$ $(x>2)$ or Ti$_2$O$_5$(OH)$_2$, the precursors of the final precipitate must also be negatively charged polymers, and the final precipitate is peroxotitanium hydrate TiO$_3$(aq), consisting of Ti$_2$O$_5$ units. Anion condensation was accompanied by an increase in the pH, hence it was much faster with increasing pH. Indeed, our previous FT-IR studies confirmed the presence of -O-O- groups of Ti$_2$O$_5$ units on the titanium surface treated with an H$_2$O$_2$ 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., it is suggested that a fraction of the Ti-O-Ti links of the Ti$_2$O$_5$ units is cleaved to the Ti-O-Ti links yielding TiO$_2$(aq). Therefore, one can expect that the Ti(IV)-H$_2$O$_2$ complexes that formed under the present chemical treatment conditions involve TiOOH(aq), TiO$_3$(aq) and TiO$_2$(aq).

In order to discuss both the formation of TiOOH(aq) and TiO$_2$(aq) (hydrated titania gel) and the dissolution of TiO$_2$(aq), a series of reactions leading to direct formation of titanium peroxides in the titanium and hydrogen peroxide system (Ti-H$_2$O$_2$) were considered on the basis of Tengvall et al.: They involve some redox reactions that the derivative species are subsequently subject to following.

\[
\begin{align*}
\text{Ti(s)} + 2\text{H}_2\text{O}_2 &\rightarrow \text{TiOOH(aq)} + \text{H}_2\text{O} + \text{H}_2\text{O}_2\text{(aq)} \\
\text{TiOOH} + \text{1/2O}_2 &\rightarrow \text{Ti(OH)}_4
\end{align*}
\]

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

\[
\text{pH} = p\text{H}_i - \log(C_{\text{Ti}})
\]

Fig. 12 compares the experimental and calculated values of the pH for the H$_6$-NH$_4$OH, H$_6$, and H$_6$-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$_2$O$_2$ reactions, hence it is necessary to take the H$^+$ consumption in eqs. (4), (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$_{\text{emp}}$, and calculated pH$_c$:

\[
\log DH^+ = -p\text{H}_c/p\text{H}_{\text{emp}}
\]

Fig. 13 plots DH$^+$ for those H$_6$ solutions on the basis of eq. (9) as a function of the treatment time. Regardless of the solution, DH$^+$ increased in the order: H$_6$-NH$_4$OH solution < H$_6$ solution < H$_6$-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 $\Delta H^+$ 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 $\Delta H^+$ and the slope of the curve for the H6 solution well agreed with those for the H6-NH$_4$OH solution.
* in the period longer than 6 h $\Delta H^+$ 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 equations (3) through (7) above are concerned, the formation of Ti(OH)$_4$ and Ti(OH)$_{4-n}$ is involved. Thus, the similarity in the change in $\Delta H^+$, 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-NH$_4$OH solutions in the range of <6 h 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 $\Delta H^+$ 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)$_{4-n}$ 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$_2$O$_2$ 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$_2$O$_2$ can be interpreted by considering that the oxidation of titania, 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$_2$O$_2$ 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$_2$O$_2$ 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 solutions$^{16-19}$ based on the chemical treatment condition of Wang et al.$^{5,6}$. Since the process of Wu
et al.\textsuperscript{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.\textsuperscript{16} and Wu et al.\textsuperscript{16-19} reported that thus-obtained crystalline titania had a high \textit{in vitro} apatite-forming ability within 1 day. However, their chemical treatment required a high concentration of H\textsubscript{2}O\textsubscript{2} (15-30 mass\%) or a low pH condition (HCl or TaCl\textsubscript{5} 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 H\textsubscript{2}O\textsubscript{2} 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.

\textit{in vitro} Apatite-forming Ability of Titania Gel Layer

After Li et al.\textsuperscript{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 H\textsubscript{2}O\textsubscript{2} could yield a titania gel involving TiOOH(aq), TiO\textsubscript{3}(aq), and TiO\textsubscript{2}(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 H\textsubscript{2}O\textsubscript{2} solution developed thin titania layers, which unfortunately could not deposit apatite unless they were heat-treated \textsuperscript{20-24} or were chemically treated with the low-temperature process for crystalline titania proposed by Wu et al.\textsuperscript{16-19} Wålivaara et al.\textsuperscript{13} reported that the chemical treatment in H\textsubscript{2}O\textsubscript{2} 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.\textsuperscript{6} suggested that thermal treatment at 400°C eliminated the Ti–O–O–Ti and TiOOH groups from the titania gel derived by H\textsubscript{2}O\textsubscript{2} treatment and promoted the formation of TiO\textsubscript{2} (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\textsuperscript{-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.\textsuperscript{6}. Contradictory results were also reported. Hayakawa et al.\textsuperscript{7} recently treated Ti chemically with H\textsubscript{2}O\textsubscript{2} containing TaCl\textsubscript{5} 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 only. They supposed 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 H\textsubscript{2}O, 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 apatite\textsuperscript{23-26}. However, the \textit{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.\textsuperscript{23} reported that the TaOH groups on a tantalum metal substrate required at least 1 week to induce apatite nucleation in SBF\textsuperscript{25}. Thus, the primary factors for favoring good \textit{in vitro} apatite-forming ability of the titania gel derived by chemical treatment with H\textsubscript{2}O\textsubscript{2} containing TaCl\textsubscript{5} 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.\textsuperscript{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.\textsuperscript{27} reported that the sodium titanate layer yielded Ti-OH groups due to ion-exchange between the sodium ions in the layer...
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and oxonium ions (H$_3$O$^+$) 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 formation.29-33 Pereira et al. suggested that the critical pore size for rapid apatite formation is larger than 2 nm, 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 silica.34-35 Peltola et al.34-36 also reported that the pore structure and thickness of sol-gel derived titanias film do not have a clear effect on apatite formation. They suggested 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 H$_6$/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.6 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 Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ (hydroxyapatite) as proposed by Wang et al. or Mao et al., 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 provide a high blood-compatible titania gel 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ treatments were smaller than that in 6 mass% H$_2$O$_2$ treatment, while the Ti(IV) dissolution from titania gel occurred in 10 mass% H$_2$O$_2$ treatment more preferentially than in 6 mass% H$_2$O$_2$ treatment. The titania gel formation was controlled not only by the H$_2$O$_2$ 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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