Surface Modification of Commercially Pure Ti Treated with Aqueous NaOH Treatment and Ethyl Alcohol Aging

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Abstract

This study evaluates the biomimetic calcium phosphate-forming abilities of commercially pure titanium (c.p. Ti) substrates treated with NaOH aqueous solution and subsequent ethyl alcohol aging before being soaked in simulated body fluid (SBF). Specimens of c.p. Ti were initially treated with 5 M NaOH at 60 °C for 24 h, resulting in the formation of a porous network structure composed of sodium hydrogen titanate (NaH2Ti3O7). The specimens were then aged in ethyl alcohol at 60 °C for 5 or 10 min, and subsequently immersed in SBF at 37 °C for 3, 7, and 14 days, respectively. The calcium phosphate-forming abilities of the c.p. Ti after a single NaOH treatment were low, but significantly increased after ethyl alcohol aging. Similarly, aging in ethyl alcohol reduced the water contact angles of the surfaces. NaOH treatment combined with ethyl alcohol aging for 5 min resulted in the greatest deposit of calcium phosphate on the c.p. Ti.

Keywords: Titanium, Alkali treatment, Ethyl alcohol aging, Calcium phosphate, Simulated body fluid (SBF)

1. Introduction

Due to its mechanical and chemical properties, commercially pure titanium (c.p. Ti) is currently the material of choice for osseointegrated implants [1]. However, c.p. Ti does not bond directly to living bones after implantation into the body [2]. Therefore, various surface modifications have been investigated in the past decade to give titanium bioactive bone-bonding ability. Modifications have been produced via plasma spraying, sputter deposition, sol-gel coating, electrophoretic deposition, and biomimetic deposition [3-10]. Coatings are very effective in terms of bioactivity improvement, but problems related to their adhesion limit their use [11]. Various treatments have been proposed to induce bioactive behavior on the surface of a metal, potentially eliminating the need for coatings. Additionally, most coating techniques (e.g., plasma spraying, sputter deposition, and micro-arc oxidation) require high-temperature or high-vacuum conditions, leading to cracks in the fabricated films during cooling [12]. Ti-based bulk metallic glasses with superior mechanical properties and a low elastic modulus are expected to be applied as a new type of biomaterial. Ti-based bulk metallic glasses developed via hydrothermal hot-pressing induces apatite formation and can bond with hydroxyapatite (HA) ceramics [13-15].

Recently, chemical modification of a biomaterial surface that can prevent the separation between the planted metal and bone tissue caused by fibrous cells has been developed. The chemically modified surface bridges the metal implant and bone by forming a direct chemical bond, making a bioactive ceramic HA coat unnecessary. The chemical treatment of titanium metals is a simple and effective technique and has been studied widely in recent years. The reagents most frequently employed in this type of treatment are NaOH [16-21] and H2O2 [22,23]. Treatment with an NaOH solution produces a sodium titanate gel layer on the surface of Ti whereas H2O2 produces a titania gel layer. Both gel layers have been reported to induce the deposition of bone-like apatite during soaking in simulated body fluid (SBF) and are thus considered bioactive.

Soaking in only NaOH has recently been found to be beneficial with respect to improving the adhesion of the coating to the substrate and enhancing the bone growth around HA-coated implants in rabbits [24]. Classical biomimetic calcium phosphate formation normally requires an immersion period of about 14 to 28 days, with replenishment with an SBF solution. Recently, efforts have been made [25-28] to accelerate this process to increase its practical use. As indicated in the results
of Ho et al., [29] the apatite-forming ability of NaOH-treated and Bioglass®-coated Ti-7.5Mo was higher than that of c.p. Ti under the same conditions. Uchida et al. [30] found that the apatite-forming ability of titanium induced by alkali treatments can be greatly enhanced by combining hot water and subsequent heat treatments. Moreover, Hsu et al. [31] found that the apatite-forming abilities of c.p. Ti and Ti-7.5Mo after a single NaOH treatment were low, but significantly increased by aging the specimens in distilled water at 80 °C for 12, 24, or 48 h. In the present study, c.p. Ti substrates were treated with NaOH aqueous solutions and ethyl alcohol aging. An SBF immersion test was performed to determine the bioactivity of the treated c.p. Ti. Apatite formation on the surface of an implant is believed to be a prerequisite for in vivo bone-bonding ability.

2. Materials and methods

The material used for this study was c.p. Ti. Flat sheets of c.p. Ti, 10 × 10 × 1 mm³ in size, were used as substrate materials. The surfaces of the metals were abraded, finishing with 2000-grit paper. The metal substrates were then ultrasonically cleaned in distilled water, acetone, and ethanol for 20 min, respectively, after which the substrate plates were again cleaned in distilled water for another 10 min. Following this, the cleaned specimens were immersed in 5 M NaOH aqueous solution at 60 °C for 24 h. The temperature was maintained using a water bath. After the 24-h incubation period, the substrates were gently washed with distilled water, and dried at 40 °C for 24 h.

For sample coding, N denotes NaOH treatment and EA denotes ethyl alcohol aging. N-EA5 and N-EA10 represent c.p. Ti samples subjected to NaOH treatment followed by ethyl alcohol aging for 5 and 10 min, respectively. After the NaOH treatment, the substrates of c.p. Ti were gently washed with distilled water, and then immersed in 20 ml of ethyl alcohol at 60 °C for either 5 or 10 min. After the respective periods of aging, the specimens were removed and immediately soaked in 20 ml of SBF to test the capability of c.p. Ti to spontaneously form a bone-like apatite layer in vitro. The SBF was prepared by dissolving reagent-grade NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂, and Na₂SO₄ in distilled water. The ionic concentrations of the SBF (vs. human plasma) are listed in Table 1 [32]. The treated substrates were immersed in SBF at 37 °C for 3, 7, and 14 days, respectively. The temperature was maintained using a water bath and the SBF was refreshed every 2 days to preserve its ion concentration. After being soaked for the selected durations, the specimens were removed from the fluid, washed with distilled water, and air-dried.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration (mM)</th>
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<tbody>
<tr>
<td>Na⁺</td>
<td>142.0</td>
</tr>
<tr>
<td>K⁺</td>
<td>5.0</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>1.5</td>
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<tr>
<td>Ca²⁺</td>
<td>2.5</td>
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<tr>
<td>Cl⁻</td>
<td>103.0</td>
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<tr>
<td>HPO₄³⁻</td>
<td>1.0</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>0.5</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>27.0</td>
</tr>
</tbody>
</table>

Table 1. Ionic concentrations (mM) of simulated body fluid compared to human blood plasma [32].

After the NaOH treatment and ethyl alcohol aging, the surfaces of the c.p. Ti specimens were examined by field-emission scanning electron microscopy (FE-SEM; JSM-6700F, JEOL, Japan) and X-ray diffraction (XRD; MXP-III, Brukers, Germany). Changes in the surfaces of the c.p. Ti samples after being soaked in SBF were determined using a SEM (S-3000N, Hitachi, Japan), and surface chemical analysis was conducted using energy-dispersive X-ray spectroscopy (EDS) in a SEM. To evaluate the ability and rate of apatite formation on the sample surface, changes in the pH values of the SBF were determined each day using an electrolyte-type pH meter (Ion 6 meter, Oakton, USA). The weight of the apatite coating on each specimen soaked for a given length of time was obtained using an electronic balance (SD-200L, Mirage, Japan) with an accuracy of ± 0.0001 g. The SBF was refreshed after each measurement. Contact-angle measurements were carried out using Millipore water on a contact angle measurement device (OCA 15, DataPhysics Instruments, Filderstadt, Germany) with SCAl20 software (Dataphysics, Germany) to evaluate the wettability of the c.p. Ti specimens after they had been subjected to various pre-treatments.

3. Results and discussion

3.1 Surface morphologies after NaOH treatment and ethyl alcohol aging

Figure 1 shows the changes in the surfaces of c.p. Ti substrates before and after NaOH treatment, and after undergoing ethyl alcohol aging at 60 °C for 5 or 10 min. The surface of the untreated c.p. Ti appears flat, with several grinding marks visible. After NaOH treatment, the surface of the c.p. Ti substrate exhibited a porous network structure that became much denser after undergoing ethyl alcohol aging.

![FE-SEM images of c.p. Ti surfaces subjected to NaOH (N) or NaOH-ethyl alcohol (N-EA) treatments for 5 or 10 min.](image-url)

Figure 2 shows the XRD patterns of the surfaces of the c.p. Ti substrates that were subjected to ethyl alcohol aging at 60 °C for 5 or 10 min, after they had been soaked in the NaOH solution. Previous studies have reported that this porous layer
could be sodium hydrogen titanate (Na$_3$H$_2$Ti$_5$O$_{12}$) [18,33]. When the specimens were aged in ethyl alcohol, the sodium ions in the sodium hydrogen titanate hydrogel were released. This is similar to the process that takes place when specimens are soaked in SBF [30,34]. Pattanayak et al. [35] found that 5.5 at.% Na was incorporated into the surface structure of the c.p. Ti after 5 M NaOH treatment, then became completely removed by subsequent water or HCl treatments. Yamaguchi et al. [18] reported that 5 M NaOH treatment led to the incorporation of 4.9 at.% Na ions into the surface structure of a Ti-15Zr-4Nb-4Ta alloy. The Na ions were completely removed by both water and 0.5 or 30 mM HCl treatments.

![Figure 2](image2.jpg)

Figure 2. XRD patterns of c.p. Ti surfaces subjected to NaOH (N) or NaOH-ethyl alcohol (N-EA) treatments for 5 or 10 min.

### 3.2 Wettability

Figure 3 shows the average water contact angles of the untreated c.p. Ti specimens as well as those treated with NaOH and subsequent ethyl alcohol aging for 5 or 10 min. Whereas distilled water contacted the untreated samples at angles of approximately 26° for c.p. Ti, after subsequent NaOH treatment, the surface became easily wetted, resulting in a very low contact angle (15°). The water contact angles of the surfaces were further reduced by aging in ethyl alcohol. The contact angles of the samples that were ethyl-alcohol-aged for 5 min were the lowest (less than 9°). Rupp et al. [36] also found that hydrophobic sand-blasted/acid-etched c.p. Ti surfaces could be made hydrophilic by storage in water or an NaCl solution. Therefore, it can be concluded that the NaOH treatment and ethyl alcohol aging greatly influenced the wettability of the c.p. Ti surfaces.

Surface wettability is one of the most important parameters affecting the biological response to an implanted material. This attribute affects protein adsorption, platelet adhesion/activation, blood coagulation, and cell and bacterial adhesion [37-40]. Highly hydrophobic surfaces seem more desirable than hydrophobic ones in view of their ability to interact with biological fluids, cells, and tissues [41,42]. In a recent animal study, Researchers found that a hydrophilic sand-blasted/acid-etched surface enhanced bone apposition during the early stages of bone regeneration [42].

![Figure 3](image3.jpg)

Figure 3. Average water contact angles of c.p. Ti surfaces subjected to NaOH (N) or NaOH-ethyl alcohol (N-EA) treatments for 5 or 10 min.

### 3.3 Ca-P precipitation on treated surfaces

Figure 4 shows SEM images of the surfaces of c.p. Ti samples that had been soaked in SBF for 3, 7, and 14 days, respectively, after NaOH treatment and subsequent periods of aging in ethyl alcohol. As can be seen, there was no new particle formation by nucleation on the surfaces that had undergone pre-treatment and soaking in SBF solution for 3 days. However, the surfaces of c.p. Ti were completely covered with a thin layer of calcium phosphate. After soaking in SBF solution for 7 and 14 days, a dense calcium phosphate layer covered the surfaces of the ethyl-alcohol-aged c.p. Ti substrates, depleting the Ca$^{2+}$ and PO$_4^{3-}$ ions from the surrounding solutions. Cracks in the calcium phosphate layer on the c.p. Ti are likely to have formed as a result of the contraction of the porous hydrated layer when the specimens were dried after being soaked in SBF. The formation of calcium phosphate layers with a similar morphology has been reported for Ti and several of its alloys [17,43,44].

![Figure 4](image4.jpg)

Figure 4. SEM micrographs of surface morphology of NaOH-treated (N) and subsequently ethyl-alcohol-aged (N-EA) c.p. Ti after soaking in SBF for 3, 7, and 14 days.
The results of the EDS analysis of the NaOH-treated and ethyl-alcohol-aged surfaces of the specimens soaked in SBF for 14 days are shown in Fig. 5. Under these conditions, all the specimens exhibited very intense peaks of Ca and P. The intensities of the Ti peaks were much lower for the c.p. Ti subjected to ethyl alcohol aging for 5 min. This result could be due to the interference from the apatite deposits that appeared after soaking, indicating that these coatings were thick enough to prevent the penetration of X-ray beams into the substrate surface.

Throughout the period of immersion in SBF, the titanium showed varying rates of weight gain, as shown in Fig. 6. These variations were due to the differential dissolution and precipitation rates of Ca and P. Once the calcium phosphate nucleation started, it was expected that there would be consistent weight gain. The calcium phosphate deposits were greater for the ethyl-alcohol-aged c.p. Ti samples than for their non-ethyl-alcohol-aged counterparts, as indicated in the measurements of weight gain (Fig. 6). Furthermore, the deposited amounts of calcium phosphate were greatest for the c.p. Ti that had been aged in ethyl alcohol for 5 min. These results are consistent with SEM (Fig. 4) and EDS analysis (Fig. 5) results.

The pH value of the SBF was found to increase gradually during the immersion of the NaOH-treated and ethyl-alcohol-aged c.p. Ti samples, thus indicating an increasing concentration of hydroxyl group (OH\(^-\)) ions. This pH approached peaks of about 8.45-8.47 on the third day for c.p. Ti samples subjected to NaOH treatment and ethyl alcohol aging. However, for samples which were not ethyl-alcohol-aged, the pH approached peaks of 8.45 on the fourth day, as shown in Fig. 7. This pH increase in the SBF occurs during dissolution due to the release of cations from the sodium titanate layer [45]. During immersion in SBF, the pH values reached their maximum after either three or four days, and subsequently decreased with further soaking, thus implying that a continuous precipitation of the bone-like apatite layer would occur thereafter. In the present experiment, the calcium phosphate precipitates began to deposit on the pre-treated c.p. Ti substrates after three or four days in SBF, which corresponds with the peak pH value attained on the third or fourth day.

It can be concluded that NaOH treatment and subsequent ethyl alcohol aging, in particular for 5 min, can improve apatite-forming ability. After extensive investigations into the mechanisms of apatite deposition, it is now widely accepted that the number of Ti-OH functional groups plays a determining role in the formation of bone-like apatite layers in SBF [46,47]. After NaOH-treated and ethyl-alcohol-aged...
titanium is immersed in SBF, a large number of negatively charged Ti-OH groups form [48-50] and can combine with the positively charged Ca\(^{2+}\) ions in the SBF. If there is a sufficient number of Ti-OH groups on the surface of the Ti, the Ca\(^{2+}\) ions can continually accumulate on the surface, gradually producing an overall positive charge. As a result, the positively charged surface combines with negatively charged phosphate ions to form calcium phosphates.

According to these results, although all samples of c.p. Ti subjected to NaOH treatment and ethyl alcohol aging show a greater apatite-forming ability than that of samples without ethyl alcohol aging, increasing the ethyl alcohol aging period (to 10 min) did not further improve apatite-forming ability. This finding is in agreement with the measured water contact angles (Fig. 3), which were lowest for samples that underwent ethyl alcohol aging for 5 min. This is further confirmed by other study that enrichment of Ti-OH groups increases the surface energy (by decreasing the contact angle) on Ti surfaces.

4. Conclusion

The biomimetic calcium phosphate-forming abilities of c.p. Ti substrates treated with NaOH aqueous solution and subsequent ethyl alcohol aging before being soaked in simulated body fluid (SBF) were evaluated. The surface of the NaOH-treated c.p. Ti substrate exhibited a porous network structure. This structure became much denser after ethyl alcohol aging. Sodium hydrogen titanate (Na\(_2\)H\(_2\)Ti\(_4\)O\(_9\)) reaction layers appeared on all c.p. Ti samples after NaOH treatment. NaOH treatments of c.p. Ti enhanced surface wettability. The water contact angles of the surfaces were further reduced after subsequent aging in ethyl alcohol. The contact angles measured after 5 min were the lowest (less than 9°). A thin layer of calcium phosphate formed on the surface of c.p. Ti that had undergone pre-treatment and subsequent soaking in SBF solution for 3 days. After soaking in SBF solution for 7 and 14 days, a dense calcium phosphate layer covered the surfaces of all the pre-treated c.p. Ti substrates.

References

The absence and presence of plasma

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