Effects of Chemical and Heat Treatments on Surface Characteristics and Biocompatibility of Titanium-Niobium Alloys

Kuo-Chih Feng¹, En-Yu Wu¹,*, Yung-Ning Pan¹ and Keng-Liang Ou²

¹Department of Mechanical Engineering, National Taiwan University, Taipei, 106 Taiwan, R. O. China
²Graduate Institute of Oral Sciences, Taipei Medical University, Taipei, 110 Taiwan, R. O. China

Alkali solution- and heat-treatments were employed to modify the surfaces of Ti-6Al-4V, Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys, and then the surface characteristics and the biocompatibility of the treated alloys were analyzed. The formation of porous surfaces that contain numerous nano-pores and irregular cracks, together with the increase in hydroxyl, TiO₂ and Nb₅O₁₅ contents in the surface layer of the treated Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys, have been found to enhance biocompatibility which was evaluated by the osteoblast cell culture in vitro. In addition, the biocompatibility of both Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys are comparable to that of Ti-6Al-4V alloy. Alkali solution- and heat-treatments are believed to enhance bone-bonding ability of both Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys, and therefore are useful for surface modification practice. [doi:10.2320/matertrans.MER2007083]

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1. Introduction

Titanium-based alloys are attractive implant materials due to their high strength-to-weight ratio, excellent mechanical properties, and acceptable bio-corrosion resistance and biocompatibility.¹⁻⁴ The Ti-6Al-4V alloy is currently one of the most widely used titanium alloys for surgically implanted parts, such as for knee, hip and shoulder replacements. However, the element V (vanadium) has been found to cause severe tissue reaction in animals.⁵⁻¹¹ Also, there is a concern with element Al (aluminum) due to its possible connection to neurological disorders and Alzheimer’s disease.⁷,¹¹⁻¹³ Therefore, synthesis of new titanium-based alloys, which are compatible with the human body, has arrived at the adoption of more biocompatible metallic alloying elements, such as Nb, Ta and Zr.⁹,¹２,¹⁴,¹⁵

The selection of materials for different components in implant prostheses depends on several factors, including design and required strength of the system in application. In addition, long-term studies have indicated that insufficient load transfer from an artificial implant to the adjacent remodeling bone may cause bone resorption and eventual loosening of the prosthetic device,¹⁶,¹⁷ a phenomenon termed “stress shielding effect”. This so-called stress shielding effect is a direct consequence of the stiffness mismatch between the implant material and the surrounding natural bone.¹⁸⁻²⁰ The tackling of this problem has evoked a number of solutions that proposed more flexible designs and the selection of relatively low modulus materials. Recent attempts in further minimizing the modulus of implant alloys had led to the development of β-type titanium alloys, such as Ti-13Nb-13Zr, Ti-15Mo-5Zr-3Al, Ti-12Mo-6Zr-2Fe, Ti-12Mo-5Ta and Ti-29Nb-13Ta-4.6Zr. The elastic moduli of the aforementioned alloys have been reduced to the range of 74–88 GPa, but the moduli are still 2–7 times as high as that of cortical bone (10–30 GPa).¹² The present authors had developed certain Ti-Nb-based alloys,²¹⁻²³, i.e., Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf, which had been shown to exhibit excellent properties combination of relatively high strength and low modulus (UTS: 893 MPa, 0.2% Proof stress: 841 MPa and E: 65 GPa for Ti-40Nb-1Hf, and UTS: 914 MPa, 0.2% Proof stress: 862 MPa and E: 62 GPa for Ti-30Nb-1Fe-1Hf), and are considered quite suitable for implants application. Kim et al.¹³,¹⁴ and Lee et al.²⁴ reported that a porous bioactive surface was formed on the titanium surfaces by alkali and heat treatments that can attain better bonding with living bone. Therefore, in this study, alkali- and heat-treatments were performed to modify the surfaces of the aforementioned two Ti-Nb alloys (Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf) and also Ti-6Al-4V, and then the surface characteristics and the biocompatibility of the treated alloys were analyzed and compared.

2. Experimental Procedures

2.1 Alloy preparation

Ti-Nb-Fe-Hf alloys were prepared using pure titanium (99.7 mass% in purity), pure niobium (99.8 mass% in purity), pure iron (99.98 mass% in purity) and pure hafnium (99.98 mass% in purity). The weighed charge materials (some 400 grams) were placed in a water-cooled copper crucible and then melted using a non-consumable tungsten electrode arc in a vacuum chamber. The melting chamber was first evacuated and then purged with argon. An argon pressure of 0.1 MPa was maintained throughout the melting process. The alloys were re-melted four more times to achieve chemical homogeneity. The chemical analyses of the Ti-alloys studied herein are listed in Table 1. The solidified Ti-Nb-Fe-Hf alloy ingots were homogenized at 1000°C (1273 K) for 6 hours at a vacuum of better than 0.27 Pa, and then hot-rolled at 750°C (1023 K) into plates with a thickness of approximately 2 mm. The final rolled specimens were again annealed at 700°C (973 K) for 1 hours, and then furnace cooled to room temperature. 

*Graduate Student, National Taiwan University. Corresponding author, E-mail: d92522004@ntu.edu.tw
were removed by sputtering with an Ar

NaOH aqueous solution at 60

treatments is

2.2 Alkali solution- and heat-treatments

The size of the specimens for alkali solution- and heat-treatments is 10 \times 10 \times 1 \text{ mm}. The specimens were first polished sequentially with SiC abrasive papers of the following grits: 80, 240, 600, 1200, 2000 and 2400, and then cleansed with acetone in an ultrasonic bath for about 10 minutes. The specimens were first soaked in 5 kmol/m³ NaOH aqueous solution at 60°C (333 K) for 24 hours, and then rinsed with deionized water and then dried at 40°C (313 K) for another 24 hours. In addition, some of the alkali solution treated specimens were heated up to 600°C (873 K) for 1 hours. The designation of specimens with different treatments is denoted in Table 2.

2.3 Analysis of surface characteristics

The surface morphologies of the treated alloys were analyzed by scanning electron microscope (SEM) and high resolution field-emission scanning electron microscope (FE-SEM). The chemical states of the surface layers were analyzed by X-ray photoemission spectroscopy (XPS) with a monochromatic Al K\alpha source (1486.6 eV) operating at 108 W (15 kV), which could examine a surface area of 400 μm in diameter. In all cases, the surface contamination layers were removed by sputtering with an Ar⁺ ion gun (3 keV, 1 μA) at an angle of 45°. X-ray powder diffractometer (XPRD) was employed to identify the phases present in the surface layers. The incident angle of the X-ray was fixed at 1°. The X-ray diffractometer with a Ni-filtered Cu K\alpha radiation source was operated at 40 kV, 150 mA, 2 deg/min scanning rate and 20~60 scanning range. Phases were identified by matching their characteristic peaks with those in the files of the Joint Committee on Powder Diffraction Standards (JCPDS). For identifying the aqueous wettability of the treated alloys, the contact angles, determined using drops of ultrapure distilled water, were measured using a FTA-32 video contact angle system (First Ten Angstrom Inc., USA) at room temperature.

2.4 Cell culture

In order to evaluate the biocompatibility of Ti-alloys without and with alkali solution- and heat-treatments, the test specimens (10 \times 10 \times 1 \text{ mm}) were cell (MG-63) cultured and then the morphology and proliferation of the cells were observed. The test specimens were placed into a 24-well polystyrene plate. Before cell culture, all the specimens were shone by ultraviolet ray (UV) from under for 24 hours. The test specimens were sterilized and washed several times with Dulbecco’s modified Eagle’s medium (DMEM, Gibco) and phosphate-buffered saline (PBS, 0.1 M, pH 7.2). The culture medium consisted of DMEM containing 10% fetal bovine serum (FBS), 100 μg/ml of streptomycin, and 100 units/ml of penicillin.

The MG-63 cell suspension with a 3 × 10⁴/cm² density was added into the plate. 500 μl culture solution and 50 μl 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) label solution were added into every culture well, and the plate was placed inside a culture chamber at 37°C in a humidified atmosphere of 95% air and 5% CO₂. The culture medium was changed every three days. The test specimens were cultured for various periods of time, i.e., 24 hours, 72 hours and 144 hours. The extent of adhesion and proliferation of MG-63 cells on the treated surfaces of various Ti-alloys were evaluated through the observations of cell morphologies by using SEM, calculating the cell count by the hemocytometer, and performing MTT assay to obtain the cell optical density (OD) by the plate reader (ELISA, DYNEA-MRX II) at λ = 595 nm.

3. Results and Discussion

3.1 Surface morphology

The surface morphologies of the non-treated and alkali solution- and heat-treated Ti-alloys are shown in Fig. 1. Note that the porous network morphology is evident on the surface of NHT-6 (Fig. 1(b)). The porous network formed on the surface of Ti-6Al-4V alloy by alkali solution- and heat-treatments is similar to that reported by Lee et al. On the other hand, irregular cracks can be observed in both NHT-40 and NHT-30 (Figs. 1(d) & 1(f)), which were induced by thermal heat during holding the specimens at a high temperature of 600°C (873 K). Similar results had also been reported by Li et al. Further analysis indicates that nano-pores of some 10~20 nm can be observed on the surfaces of NHT-40 and NHT-30 under relatively high magnifications by FE-
SEM (Fig. 2), which are substantially smaller than those in Ti-6Al-4V alloy (50~100 nm). The presence of nano-pores that results in an increase in surface area is believed to improve bioactivity and therefore the rate of bone formation.

3.2 Wettability analysis

The results regarding the aqueous wettability test are shown in Fig. 3. It reveals that the contact angle is substantially decreased as Ti-alloys were modified with alkali solution- and heat-treatments. The results clearly indicate that the wettability of the Ti-alloys is enhanced by surface modification. It has been indicated that the wettability corresponds to the surface bonding energy between titanium and the body fluid, and also a hydrophilic surface not only can enhance interaction between implant surface and the biologic environment, but also can promote cell proliferation, adhesion and differentiation. Based upon the above arguments, the alkali solution- and heat-treated Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys can be expected to promote the interaction between the biological fluid and the implant surface, and therefore the biocompatibility is improved.

3.3 XRD analysis

The phases in the oxide layer formed due to surface modification were analyzed by XRD method, and the results are shown in Figs. 4–6. Figure 4 displays the XRPD spectra of T-6, NT-6 and NHT-6. For alloy T-6, the primary phase is α-Ti. For alloy NT-6, new peaks appeared around 23°~29° and 48°. According to a previous study, these peaks correspond to an amorphous sodium titanate hydrogel layer. For alloy NHT-6, the sodium titanium oxide (Na$_2$Ti$_6$O$_{13}$) and rutile formed on the surface. The above observations reveal that alkali solution treatment alone can form a amorphous phase of sodium titanate hydrogel, while alkali solution- and heat-treatments can transform a amorphous phase into a crystalline phase, which is in agreement with the findings of some previous works.

Figure 5 displays the XRPD spectra of T-40, NT-40 and NHT-40. The analysis results indicate that for both T-40 and NT-40, the phase present is entirely of β-Ti. For alloy NHT-40, only the rutile peak can be detected. Figure 6 displays the XRPD spectra of T-30, NT-30 and NHT-30. The analysis results indicate that for both T-30 and NT-30, the phase present is entirely of β-Ti. However, for alloy NHT-30, alkali
solution- and heat-treatments tend to promote the formation of rutile and Nb$_2$O$_5$ (monoclinic).

The results reveal that alkali solution treatment along does not causes any detectable change in the surface structure in both Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf, while alkali solu-
tion- and heat-treatments can induce the formation of rutile for Ti-40Nb-1Hf and rutile and Nb$_2$O$_5$ for Ti-30Nb-1Fe-1Hf.

It is generally accepted that titanium exhibits its excellent biocompatibility via its very stable and corrosion resistant oxide layer. The excellent tissue response to titanium is believed to be related to the chemical and biochemical properties of titanium oxides on the titanium surface.³₀ Huang et al.²₉ had demonstrated that the blood compatibility of titanium increases with increasing rutile content of titanium oxide. In addition, the presence of niobium oxides such as Nb$_2$O$_5$, had been found to possess excellent
biocompatibility and corrosion resistance. Therefore, alkali solution- and heat-treated Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys can be expected to possess improved biocompatible and corrosion resistance.

### 3.4 XPS analysis

Chemical bonding states of alloy surfaces were studied by XPS for the Ti-alloys with and without surface modification. By using a computer assisted Gaussian-Lorentzian peak model to curve fit the spectra, the results concerning Ti 2p are shown in Figs. 7–9 for Ti-6Al-4V, Ti-40Nb-1Hf, and Ti-30Nb-1Fe-1Hf, respectively. For untreated Ti-alloys (T-6, T-40 and T-30), the binding energies at 453.2 eV, 457.8 eV, and 458.33 eV correspond to Ti$^{0+}$, Ti$^{3+}$, and Ti$^{4+}$2p$_{3/2}$ emissions, respectively. In addition, binding energies at 463.1 eV and 464 eV also correspond to Ti$^{3+}$ and Ti$^{4+}$2p$_{1/2}$ emissions, respectively. It is clear that Ti$^{0+}$, Ti$^{3+}$ and Ti$^{4+}$ chemical states are present in the surface oxide layers of the untreated Ti-alloys.

Regarding the effect of alkali solution- and heat-treatments, for NHT-6, the valence states of Ti$^{3+}$ and Ti$^{4+}$ remain more or less unchanged (Fig. 7). For NHT-40 (Fig. 8), the valence state of Ti$^{4+}$ increases, while Ti$^{3+}$ does not change much, implying that the proportion of TiO$_2$ increases, but Ti$_2$O$_3$ remains unchanged. On the other hand, the valence state of Ti$^{3+}$ (Ti$_2$O$_3$) increases substantially, but Ti$^{4+}$ (TiO$_2$) decreases slightly for NHT-30, as shown in Fig. 9.

The binding energies of the primary XPS peaks are listed in Table 3. From Table 3, the O 1s spectrum of NHT-6 has four binding energies, i.e., 529.7 eV (TiO$_2$), 530.3 eV (V$_2$O$_5$), 531.6 eV (Ti-OH) and 532 eV (OH$^-$). The Al 2p and V 2p are absent in alloy NHT6, due to the formations of Al$_2$O$_3$ and V$_2$O$_5$ on the surface. Therefore, for Ti-6Al-4V, the formations of Al$_2$O$_3$ and V$_2$O$_5$ by alkali solution- and heat-treatments can be expected to alleviate the harmful effects of Al and V by preventing these two elements from dissolving into the human body.

The O 1s spectrum of NHT-40 has four binding energies, i.e., 530.2 eV (TiO$_2$), 530.7 eV (Nb$_2$O$_5$), 531.5 eV (H$_3$O$_2$Nb$_2$O$_5$) and 532 eV (OH$^-$). The O 1s spectrum of

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ti$^{4+}$ 2p$_{3/2}$ (eV)</th>
<th>Ti$^{3+}$ 2p$_{3/2}$ (eV)</th>
<th>O 1s (eV)</th>
<th>Al$^{3+}$ 2p$_{3/2}$ (eV)</th>
<th>V$^{5+}$ 2p$_{3/2}$ (eV)</th>
<th>Nb$^{5+}$ 3d$_{3/2}$ (eV)</th>
<th>Na 1s (eV)</th>
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</thead>
<tbody>
<tr>
<td>T-6</td>
<td>458.33</td>
<td>457.8</td>
<td>530.3</td>
<td>74</td>
<td>517.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>NHT-6</td>
<td>457.8</td>
<td>456.9</td>
<td>529.7, 530.3, 530.8, 531.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1072.5</td>
</tr>
<tr>
<td>T-40</td>
<td>458.33</td>
<td>457.8</td>
<td>530, 531.3</td>
<td>—</td>
<td>—</td>
<td>206.9</td>
<td>—</td>
</tr>
<tr>
<td>NHT-40</td>
<td>458.33</td>
<td>457.8</td>
<td>530.2, 530.7, 531.5, 532</td>
<td>—</td>
<td>—</td>
<td>206.9</td>
<td>1071.8</td>
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<tr>
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<td>458.33</td>
<td>457.8</td>
<td>530, 531.3</td>
<td>—</td>
<td>—</td>
<td>206.9</td>
<td>—</td>
</tr>
<tr>
<td>NHT-30</td>
<td>458.33</td>
<td>457.8</td>
<td>530.2, 531.3, 531.5, 532</td>
<td>—</td>
<td>—</td>
<td>206.9</td>
<td>1071.8</td>
</tr>
</tbody>
</table>

Fig. 7 XPS spectra of Ti 2p for T-6 and NHT-6 alloys.

Fig. 8 XPS spectra of Ti 2p for T-40 and NHT-40 alloys.

Fig. 9 XPS spectra of Ti 2p for T-30 and NHT-30 alloys.
NHT-30 also has four binding energies, i.e., 530.2 eV (TiO\(_2\)), 531.3 eV (Nb\(_2\)O\(_5\)), 531.5 eV (H\(_2\)O\(_2\)Nb\(_2\)O\(_5\)) and 532 eV (OH\(^-\))\(^{39}\). The results indicate that TiO\(_2\), Nb\(_2\)O\(_5\) and OH groups will form on the surfaces of the treated Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys.

The presence of the OH groups on the surfaces of the Ti-alloys had been reported to enhance cell adhesion and spreading,\(^{34}\) and also to promote bone-like apatite nucleation.\(^{30}\) Therefore, the presence of OH groups together with the formations of TiO\(_2\), Nb\(_2\)O\(_5\) in the alkali solution- and heat-treated Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys should provide with a bioactive surface layer for bone healing.

### 3.5 Cell culture

#### 3.5.1 Cell proliferation

Results of MTT assay on the alloys being cultured with cells are presented in Fig. 10. The optical density (degree of cell attachment) increases with increasing culture time for the three alloys investigated. Little difference in optical density was obtained between untreated and treated Ti-alloys for the cases of 24 and 72 hours of culture time. While, for the case of 144 hours culture time, the results show that more cells attach onto the treated Ti-alloys than the untreated Ti-alloys. However, the extent of cell attachment is similar for the three Ti-alloys studied.

The effect of alkali solution- and heat-treatments on the cell count as a function of culture time is shown in Fig. 11. Little difference in cell number can be realized among three Ti-alloys investigated at a shorter culture time of 24 hours. However, for the culture times of 72 hours and 144 hours, the differences in cell number are apparent between the untreated and the treated Ti-alloys, with the latter having higher cell counts.

#### 3.5.2 Cell morphology

The SEM observations of cell morphology of Ti-6Al-4V without and with surface modification after culturing for 144 hours are shown in Fig. 12(a) and Fig. 12(b), respectively. For T-6, the cells exhibit good adhesion as shown in Fig. 12(a). For NHT-6, as can be observed in Fig. 12(b), the treated surface is almost completely covered by the cells. Moreover, the filopods of the cells that attach on the porous surface is evident in Fig. 13.

Figure 12(c) and Fig. 12(d) show the cell morphologies of T-40 and NHT-40, respectively, after culturing for 144 hours. For T-40, as indicated in Fig. 12(c), the cells have flattened with numerous filopods. However, more cells attach onto the NHT-40 (Fig. 12(d)) when compared to T-40, implying that the cell activity in NHT-40 is higher than that in T-40.

The cell morphologies after culturing for 144 hours for T-30 and NHT-30 are shown in Fig. 12(e) and Fig. 12(f), respectively. For T-30, as indicated in Fig. 12(e), the cells attach and elongate but have not flattened significantly. After alkali solution- and heat-treatments, the cells have flattened and spread substantially and have almost completely covered the surface, as shown in Fig. 12(f).

As stated earlier, porous network surfaces containing cracks and numerous nano-pores were formed when both Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys were modified with alkali solution- and heat-treatments. In addition, rutile (TiO\(_2\)), Nb\(_2\)O\(_5\) and OH groups can be detected in the treated alloys. These specific surface characteristics have been found to improve wettability and also to promote cell proliferation, adhesion and spreading. Hence, the treated Ti-alloys are believed to possess better biocompatibility and bone-bonding ability than Ti-alloys without surface modification. The present results are in accordance with the findings in the literature.\(^{6,16,19,30,37}\)

### 4. Conclusions

The previously developed titanium-niobium alloys—Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys—exhibit relatively high strength and low elastic modulus and are considered quite suitable for implants application. This study employed alkali solution treatment and heat treatment to modify the surfaces of Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys together with the currently widely used Ti-6Al-4V alloy, and then the biocompatibility of the surface treated alloys was analyzed by means of osteoblast cell culture in vitro. The
results indicate that porous surfaces were obtained for Ti-Al-4V, while surfaces with irregular cracks that contain numerous nano-pores were obtained for Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys, after surface modification. In addition, alkali and heat treatments enhance the growth of TiO$_2$ (rutile), Na$_2$Ti$_6$O$_{13}$ and Ti-OH in the surface layer of Ti-6Al-4V, and TiO$_2$ (rutile), Nb$_2$O$_5$ and OH groups in Ti-40Nb-1Hf, while Ti$_2$O$_3$, Nb$_2$O$_5$ and OH groups in Ti-30Nb-1Fe-1Hf. The formation of porous surface structure together with the increase in OH groups, TiO$_2$, Ti$_2$O$_3$ and Nb$_2$O$_5$ in the surface layer owing to surface modification have been found not only to improve wettability, but also to enhance the proliferation, adhesion and spreading of the osteoblast-like cells tested \textit{in vitro}. In summary, the biocompatibilities of Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys are improved after alkali solution- and heat-treatments, and also the activity of

Fig. 12 SEM morphologies of osteoblast-like cells after culturing for 144 hours on (a) T-6, (b) NHT-6, (c) T-40, (d) NHT-40, (e) T-30, and (f) NHT-30.

Fig. 13 SEM image (high magnification) of NHT-6.
osteoblast-like cells of the aforementioned two alloys have been found to be similar to that of Ti-6Al-4V. Alkali solution- and heat-treatments are believed to enhance bone-bonding ability of both Ti-40Nb-1Hf and Ti-30Nb-1Fe-1Hf alloys, and therefore are useful for surface modification practice.

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