***In vitro* biomimetic deposition of apatite on alkaline and heat treated Ti6Al4V alloy surface**

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Abstract. Titanium alloy (Ti6Al4V) substrates, having the ability of biomimetic calcium phosphate-based materials, especially hydroxyapatite deposition in a simulated body fluid (SBF) means of chemical treatment (alkaline treatment) and subsequent heat treatment, was studied. The effects of alkaline treatment time, concentration and heat treatment temperature on the formation of calcium phosphate (carbonate–hydroxyapatite) on Ti6Al4V surface were examined. For this purpose, the metallic substrates were treated in 0, 5 and 10 M NaOH solutions at a temperature of 60 or 80°C for 1 and 3 days. Subsequently the substrate was heat-treated at 500, 600 and 700°C for 1 h for consolidation of the sodium titanate hydrogel layer. Finally, they were soaked in SBF for 1 and 3 days. The substrate surfaces were characterized by the techniques commonly used for bulk material such as scanning electron microscopy (SEM) and thin film X-ray diffraction (TF-XRD). With regard to the SEM and TF-XRD results, the optimum process consists of 3 days soaking in 5 M NaOH in 80°C and subsequent heat treatment at 600°C for 1h. It is worth mentioning that the results showed that the apatite formed within 3 days on the specimen surfaces, however, there was no sign of apatite formation in the control samples (without alkaline and heat treatment) which was treated for up to 3 days immersion in SBF.

Keywords. Ti6Al4V; apatite; biomimetic deposition; alkaline; heat treatment; SBF.

1. Introduction

Hydroxyapatite (HA: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is an important material for bone and tooth implants, not only because its chemical composition is similar to that of bone tissues, but also it chemically bonds to bones. Owing to the inferior mechanical properties of HA, its application has been limited in clinics (Liu *et al* 2005). On the other hand, titanium (Ti) and its alloys, Ti6Al4V, for example, are the materials of choice for most dental and orthopedic applications (Shibata *et al* 2003). The many advantages of these materials include biocompatibility, good resistance to corrosion, and excellent mechanical properties (Bigi *et al* 2005). However, titanium based materials are unfortunately not bioactive materials and generally encapsulated after implantation into the living body by fibrous tissue that isolates them from the surrounding bone (Yan and Davies 1998; Nishiguchi *et al* 1999). Therefore, coating titanium and its alloys with HA and other bioactive ceramic materials including surface modification becomes a popular method for providing them with a bone-bonding ability (Nishio *et al* 2001; Costantini *et al* 2002). These over-coatings could bond to living bone without forma-

tion of fibrous tissue. These materials create a bone-like apatite (carbonate–hydroxyapatite) layer on their surface after implantation (Jonášová *et al* 2004). Therefore, apatite formation is believed to be the main requirement for bone-bonding ability of materials in our understanding of the bioactive behaviour. The formation of a bone-like apatite surface layer can be reproduced in an acellular simulated body fluid (SBF) with ion concentration nearly equal to those of inorganic part of human blood plasma (Jonášová *et al* 2002).

Various methods of coating the surface of titanium and its alloys have been tested (de Groot *et al* 1987; Klein *et al* 1991) in order to combine the mechanical properties of

Table 1. Composition of SBF and the inorganic part of human blood plasma (mmol/l) (Teixeira *et al* 2004).

Ion	Plasma (mmol/l)	SBF (mmol/l)
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{+2}	1.5	1.5
Ca^{+2}	2.5	2.5
Cl^-	103.0	147.8
HCO_3^-	27	4.2
HPO_4^{-2}	1.0	1.0
SO_4^{-2}	0.5	0.5

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Figure 1. TF-XRD patterns for the specimens subjected to 0, 5 and 10 M NaOH treatment at 60°C for (a) 1 and (b) 3 days (T, titanium and S, sodium titanate).

Figure 2. TF-XRD patterns for the specimens subjected to 0, 5 and 10 M NaOH treatment at 80°C for (a) 1 and (b) 3 days (T, titanium and S, sodium titanate).

metals with bone-bonding ability of bioactive ceramic materials. Titanium alloys coated with plasma-sprayed HA is currently used for orthopedic applications. By this method, HA powder is heated at extremely high temperatures and deposited on the metal surface with very high velocity.

Recently, several groups have focused their attention on low temperature deposition methods such as electrophoretic (Wei *et al* 1999), electrochemical (Monma 1994) and biomimetic (Jonášová *et al* 2004). The biomimetic process is a physicochemical method in which a substrate is soaked in a solution that simulates the physiologic conditions (SBF), for a period of time, long enough to form a desirable layer of calcium phosphate on the substrate surface. In contrast to plasma spraying, biomimetic methods offer the possibility to cover complex shaped

implants and elaborate on films of different Ca-P phases (Kokubo *et al* 1989; Barrere *et al* 1999). In recent years, different metal pretreatments, such as alkaline treatment (Kim *et al* 1997), acid treatment (Wen *et al* 1998), have been used for accelerating and enhancing the biomimetic coating activity.

In this study, Ti6Al4V substrates were provided with *in vitro* apatite-forming ability by means of combined chemical (alkaline) and heat treatment.

2. Materials and methods

2.1 Sample preparation

Thirty-six Ti6Al4V (ASTM [American Society for testing of Materials] F620: standard specification for alpha plus

Figure 3. Surface morphologies (by SEM) for the specimens subjected to (a) 0 (control group), (b) 5 and (c) 10 M NaOH treatment at 60°C for 3 days.

beta titanium alloy forgings for surgical implants) discs each of 20 mm diameter and 5 mm thickness were polished with 200-, 400- and 600-grit silicon carbide (SiC) paper. Then these discs were cleaned by successive ultrasonic treatments for 15 min in acetone first, secondly, in 70% ethanol, and finally in double distilled water, and subsequently the discs were dried in air. The discs were divided into three groups (12 discs per group) and subjected to the following treatment: (i) control group without any treatment, (ii) the alkaline and heat treated samples and (iii) the alkaline and heat treated samples that were post-heated at various temperatures.

2.2 Alkaline treatment

The Ti6Al4V alloy discs (second group) were soaked in NaOH solution at a concentration of 5 and 10 M, respectively. Two treatment temperatures were used (60 and 80°C) in which the specimens were treated at either temperature for 1 or 3 days. Following the treatment, the speci-

mens were slowly washed with distilled water and dried at 40°C in an electric oven for overnight.

2.3 Heat treatment

The Ti6Al4V alloy discs (third group) were alkaline-treated with 5 and 10 M NaOH at 60°C and 80°C for 1 and 3 days, after which they were washed and dried as described in the previous section. The specimens were then heat-treated at 500, 600 or 700°C for 1 h in a Ni–Cr electrical furnace in air and cooled to ambient temperature in the furnace.

2.4 Preparation of SBF

The SBF solution was prepared by dissolving reagent-grade NaCl, KCl, NaHCO₃, MgCl₂·6H₂O, CaCl₂ and KH₂PO₄ into distilled water and buffered at pH = 7.25 with trishydroxymethyl aminomethane (TRIS) and HCl 1N (1 normal

Figure 4. Surface morphologies (by SEM) for the specimens subjected to 5 M NaOH treatment at (a) 60°C for 1 day, (b) 60°C for 3 days, (c) 80°C for 1 day and (d) 80°C for 3 days.

(N) HCl = 36.5 g/L) at 37°C. Its composition is given in table 1 and is compared with the human blood plasma. The discs were then soaked in 30 ml SBF solution at 37°C for 1 or 3 days.

3. Surface characterization

The morphological investigations of the specimens before and after alkaline and heat treatment and soaking in SBF were performed by using scanning electron microscope (SEM). The specimens were coated with a thin layer of gold (Au) (thickness = 20 nm) by sputtering process for better observation of surface morphology of specimens

before taking images from SEM. The effects of alkaline treatment on the surface of the substrate and the structure of the titanium substrate, gel layer and bone-like apatite coatings obtained were analysed by thin film X-ray diffraction (TF-XRD). All specimens were examined at 2θ Bragg angles from 20° to 60° at a scanning speed of 0.02°/min.

4. Results and discussion

Figures 1 and 2 show the TF-XRD patterns of the surfaces of the NaOH-treated Ti6Al4V alloy subjected to heat treatment at various temperatures for various time periods.

Figure 5. TF-XRD patterns for the specimens subjected to 5 M NaOH treatment at 80°C for 3 days without heat treatment (N-HT) or heat-treated at 500, 600 or 700°C for 1 h, and soaked in SBF for (a) 0, (b) 1 and (c) 3 days (R, rutile; T, titanium; S, sodium titanate; O, apatite and U, unknown).

Assignments of the main diffraction peaks are given in figures 1 and 2. New broad diffraction peaks appeared around 23–30° and 47–49° in 2θ after the NaOH treatment. These peaks can be ascribed to an amorphous (partially crystalline) or a microcrystalline phase. Also these peaks can be attributed to the sodium titanate hydrogel layer formed by alkaline treatment which is responsible for apatite formation (Kokubo *et al* 1989). The leaching of Ti6Al4V in NaOH does not seem to completely dissolve the passive oxide layer (generally, titanium and its alloys are covered with a passive oxide layer [TiO₂]). Upon exposure to NaOH, this passive layer partially dissolves to form HTiO³⁻. Simultaneously, titanium is hydrated to form HTiO³⁻·*n*H₂O. These negatively charged groups react with positively charged alkali ions (Na⁺) to produce an alkali titanate hydrogel layer (sodium titanate hydrogel) on the alloy surface (Takadama *et al* 2001).

At the same treating temperature and period of time, the sodium titanate peak heights increased with increasing concentration of NaOH treatment solution. This is because more Na⁺ ions are incorporated into the metal surface, and the thickness of the gel layer increases with

increasing concentration of the NaOH solution, alkaline-treating time, and alkaline-treating temperature. However, it was found that in higher concentration of more than 10 M NaOH, thick sodium titanate film peeled off either during alkaline treatment itself or later during water rinsing. Similar intensities of sodium titanate peaks were detected for all specimens treated with 5 or 10 M NaOH at 80°C for 3 days. Thus, the least aggressive treatment resulting in the thickest layer of sodium titanate was to soak in 5 M NaOH solution at 80°C for 3 days among the trials of this experiment.

Figure 3 shows SEM photographs of the surfaces of control specimen and the NaOH-treated Ti6Al4V alloy subjected to 0, 5 and 10 M NaOH treatment at 60°C for 3 days. The SEM photograph revealed that the control specimen had a smooth surface texture with abrasive marks (figure 3a). In contrast, the alkaline-treated and the alkaline- and heat-treated titanium alloys had porous surfaces. At the same alkaline-treating temperature, a much porous structure was observed with increasing treatment time (figures 3b, c). At a constant alkaline concentration, more homogeneously distributed porous surface structure

Figure 6. Surface morphologies (by SEM) for the specimens subjected to 5 M NaOH treatment: (a) without heat treatment (N-HT) and heat-treated at (b) 500, (c) 600 and (d) 700°C for 1 h and soaked in SBF for 1 day.

was observed for the specimens treated at 80°C than for those treated at 60°C (see figure 4).

After soaking the specimens in SBF, it was clearly observed in the XRD patterns (figure 5) that apatite was formed on the surface of the specimens treated with 5 M NaOH at 80°C for 3 days when soaked in SBF for 1 day, either with or without heat treatment at 500 or 600°C for 1 h. However, apatite was not formed on the surface of the specimens which was heat-treated at 700°C. The lack of apatite formation on these specimens may be attributed

to the surface structure-changes produced by the heat treatment. The Na^+ ions from the amorphous layer during SBF soaking exchange with H_3O^+ ions produced in the surrounding fluid resulting in Ti-OH layer formation (Kokubo 1996; Takadama *et al* 2001). Simultaneously, with increasing pH the apatite nucleation gets accelerated on increasing the supersaturation of the solution with respect to apatite. Calcium ions (Ca^{2+}) are incorporated in the hydrated Ti-OH layer. The positively charged Ca^{2+} may act as nucleation sites for carbonate-hydroxyapatite

Figure 7. Surface morphologies (by SEM) for the specimens subjected to 5 M NaOH treatment (a) without heat treatment (N-HT) and heat-treated at (b) 500, (c) 600 and (d) 700°C for 1 h and soaked in SBF for 3 days.

by attaching themselves to the negatively charged (PO_4^{3-}) and (CO_3^{2-}) to form Ca-P enriched surface layer which crystallizes to bone-like apatite (carbonate-hydroxyapatite) (Jonášová *et al* 2004). The apatite formation on chemically treated Ti6Al4V alloy seems to be similar to that on bioactive glasses. The sodium ions (Na^+) released from the glass substrate via exchange with the H_3O^+ or H^+ ions in the SBF form the Ti-OH groups on their surfaces (Yan *et al* 1997; Kim *et al* 2003; Teixeira *et al* 2004). This was described to induce apatite nucleation via incorporation of Ca^{2+} or increasing the OH^- concentration. The consumption of H_3O^+ causes an increase in the pH concentra-

tion and an increase in apatite forming (Hench 1991). However, at a relatively high sintering temperature (700°C), the surface structure becomes more stable and less sodium ions are released from the substrate, therefore, less TiOH groups were formed (Yan *et al* 1997).

At room temperature (25°C), many TiOH groups were found on the surface of the specimens, and hence, apatite was easily formed with large size particles. However, it seems that they were weakly bonded to the substrate. Figures 6 and 7 show SEM photographs of Ti6Al4V alloy treated with 5 M NaOH solution at 80°C for 3 days, without heat treatment or heat-treated at 500, 600 or

700°C for 1 h, and soaked in SBF for 1 and 3 days. Relatively small apatite crystals were formed on the specimens which were heat-treated at both 500 and 600°C. This formation might be caused due to the reduction of the number of TiOH groups on the surface of the heat-treated specimens. However, apatite coverage on the heat treated specimens at 600°C is more than that at 500°C.

According to above explanations, it was determined that the best treatment conditions for Ti6Al4V specimens were immersed in 5 M NaOH solution at 80°C for 3 days followed by heat treatment at 600°C for 1 h.

5. Conclusions

Titanium alloy (Ti6Al4V) treated in NaOH can form apatite after soaking in SBF. It was found that after soaking in SBF, the Ti–OH groups formed on the alloy via exchange of Na^+ ions with H_3O^+ ions which induced apatite formation indirectly by forming an amorphous sodium titanate layer on the Ti6Al4V surface. Therefore, alkaline and heat treatment are effective pre-treatment methods for apatite formation on the Ti6Al4V surface with optimum conditions of 3-day soaking in 5 M NaOH solution at 80°C and then heat treatment at 600°C for 1 h.

Finally, it is worth mentioning that Ti6Al4V shows a fairly high apatite-forming ability in a body-like environment. Hence, it has a high bone-bonding ability, as well as mechanical stability of its surface when subjected to a NaOH treatment and subsequent heat treatment to form an amorphous sodium titanate layer on its surface. Thus, treated Ti6Al4V is believed to be useful as a bone substitute.

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