

## Effect of ethyl alcohol aging on the apatite formation of a low-modulus Ti-7.5Mo alloy treated with aqueous NaOH

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**Abstract.** The purpose of this experiment was to evaluate the apatite-formation abilities of low-modulus Ti-7.5Mo substrates treated with NaOH aqueous solutions and subsequent ethyl alcohol aging before soaking them in simulated body fluid. Specimens of Ti-7.5Mo 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. Afterwards, the specimens were aged in ethyl alcohol at 60 °C for 5 or 10 min, and subsequently immersed in simulated body fluid at 37 °C for 3, 7 and 14 days. Ethyl alcohol aging significantly increased the apatite-forming abilities of Ti-7.5Mo. The amount of apatite deposited on the Ti-7.5Mo after NaOH treatment and subsequent ethyl alcohol aging was much greater, especially after the Ti-7.5Mo specimens were aged for 5 min. Due to its excellent combination of bioactivity, low elastic modulus and low processing costs, the Ti-7.5Mo treated with NaOH aqueous solutions and subsequently aged in ethyl alcohol has promising heavy load-bearing applications.

**Keywords:** Titanium alloys; Alkali treatment; Ethyl alcohol aging; Apatite; Simulated body fluid

### 1. Introduction

Titanium and its alloys are widely used in biomedical implants like artificial hip and knee joints because they possess such favorable properties as good ductility, tensile and fatigue strength, high corrosion resistance, and excellent biocompatibility (Lu *et al.* 2005). The thin natural oxide layer that characterizes the surface of implants made from titanium and its alloys limits ion release and reactivity, meaning they are close to inert and nearly biocompatible. On the other hand, this surface inactivity often causes the development of a layer of fibrous tissue around the implanted device, hampering bone integration (Ratner 2001). Much attention is being focused on improving the bioactivity of these metals using techniques such as plasma spraying, plasma implantation, and chemical treatments (Barrère *et al.* 2003, Ong *et al.* 1992, Ducheyne *et al.* 1990, Albayrak *et al.*

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2008, Boyd *et al.* 2008, Im *et al.* 2007, De Groot *et al.* 1987 and Klein *et al.* 1991). One of the most widely accepted and commercialized bioactive coating materials is plasma-sprayed hydroxyapatite (HA) (De Groot *et al.* 1987 and Klein *et al.* 1991). Although many favorable clinical results have been reported, there have been several problems with its porosity, low fatigue strength, degradation, and delamination during long-term implantation (Yee *et al.* 1999, McNally *et al.* 2000, Geesink *et al.* 1995 and Moroni *et al.* 1998).

Several surface modifications of chemical treatments have been proposed in literature in order to promote osteointegration of titanium implants. One of these is the Kokubo treatment, which uses samples soaked in NaOH solution (Kokubo *et al.* 1996, Kim *et al.* 1996 and Yamaguchi *et al.* 2011). When titanium is treated with NaOH aqueous solution, a thin sodium titanate layer can form on its surface. A bone-like apatite layer may form after the NaOH-treated metal has been soaked in simulated body fluid (SBF). This indicates that the surface of treated titanium has bioactivity because apatite formation on the surface of an implant is believed to be a prerequisite for *in vivo* bone-bonding ability (Nishiguchi *et al.* 1999).

Among the traditional metallic biomaterials, Ti-6Al-4V ELI has become the most widely used prosthesis material to date due to its good mechanical properties, ability to resist corrosion, and its biocompatibility (Lautenschlager *et al.* 1993). But there are increasing concerns regarding the stress shielding effect and biological safety of titanium as used in implant devices; it can be cytotoxic and induce allergic reactions. The former may result in osteoporosis of the surrounding bone because an implant with a high modulus carries most of the applied stress. The latter, which is related to the cytotoxic Al, V or Ni ions released from the implant into the surrounding human body fluid, may cause long-term biological problems (Okazaki *et al.* 1998 and Ahmed *et al.* 1995). In a previous study, a new  $\alpha''$ -phase Ti-7.5Mo alloy with a lower elastic modulus was developed for biomedical applications (Ho *et al.* 1999). This Ti-7.5Mo alloy also exhibits a better strength/modulus combination and excellent corrosion resistance (Ho 2008 and Ho 2008), as well as good biocompatibility (Lin *et al.* 2007). Its strength/modulus ratio is apparently higher than those of 316 L, Co-Cr-Mo, Ti-6Al-4V ELI and most of the other Ti alloys that can potentially be used in the making of medical implants. However, as with other biomedical titanium alloys, it remains a bioinert material.

Classical biomimetic calcium phosphate coatings normally require an immersion period of approximately 14 to 28 days, with replenishment from an SBF solution. Over the past few years, efforts have been made to accelerate this process to increase its practical utility. As indicated in the results of Ho *et al.* (2010), the apatite-forming ability of the NaOH-treated and Bioglass<sup>®</sup>-coated Ti-7.5Mo was higher than that of commercially pure titanium (c.p. Ti) under the same condition. Uchida *et al.* (2002) 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.* (2011) proposed that while the apatite-forming abilities of the Ti-7.5Mo achieved by a single NaOH treatment were low, they were significantly increased by aging in distilled water at 80 °C for 12, 24 or 48 h. Furthermore, the surface of HCl and NaOH-treated Ti-7.5Mo alloy has a much higher apatite-formation ability (Hsu *et al.* 2010).

In the present study, attempts were made to assess the effect of varied ethyl alcohol-aging times on the apatite-forming ability of Ti-7.5Mo that has been subjected to NaOH-ethyl alcohol treatment. Moreover, high heat treatments were avoided because the metastable  $\alpha''$ -phase Ti-7.5Mo alloy is extremely sensitive to temperature changes. Furthermore, a high-temperature treatment process tends to result in the crystallization of the hydrogel layer, thereby reducing ionic activity and decreasing the rate of apatite nucleation and formation (Rohanizadeh *et al.* 2004,

Faure *et al.* 2009 and Chen *et al.* 2007).

## 2. Materials & methods

The material used for this study was a Ti-7.5Mo alloy (wt.%). The materials were prepared from raw titanium (99.7% pure) and molybdenum (99.95% pure) using a commercial arc-melting vacuum-pressure-type casting system. To improve their chemical homogeneity, the ingots of approximately 15 g each were melted five times, for about 55 sec each time. Prior to casting, the ingots were again melted. The difference in pressure between the melting chamber and casting chamber allowed the molten alloy to instantly drop into a graphite mold at room temperature.

In this study, the cast alloys were sectioned by using a Buehler Isomet low-speed diamond saw to obtain specimens. Flat sheets of Ti-7.5Mo,  $10 \times 10 \times 1 \text{ mm}^3$  each, were used as substrate materials. The surfaces of the metals were abraded, finishing with a 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 cleaned in distilled water for another 10 min. The cleaned specimens were immersed in 5 M NaOH aqueous solutions at 60 °C for 24 h. The temperature was maintained using a water bath. After the NaOH treatment, the substrates of Ti-7.5Mo were gently washed with distilled water, and then immersed in 20 ml of ethyl alcohol at 60 °C for either 5 or 10 min. To save space in the following sections of the paper, 'N' will stand for 'NaOH treatment' and 'EA' will stand for 'ethyl alcohol aging'. Moreover, N-EA5 and N-EA10 represent the Ti-7.5Mo sample subjected to NaOH treatment and followed by ethyl alcohol aging for 5 and 10 min, respectively.

After the respective periods of ethyl alcohol aging, the specimens were removed and immediately soaked in 20 ml SBF to test the capability of Ti-7.5Mo to spontaneously form a bonelike apatite layer *in vitro*. The SBF was prepared by dissolving reagent grade NaCl, NaHCO<sub>3</sub>, KCl, K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, MgCl<sub>2</sub>·6H<sub>2</sub>O, CaCl<sub>2</sub>, and Na<sub>2</sub>SO<sub>4</sub> in distilled water. The ionic concentrations of the SBF (vs. human plasma) are listed in Table 1 (Kokubo *et al.* 2006). The treated substrates were immersed in this SBF at 37 °C for 3, 7 and 14 days, respectively. The temperature was maintained using a water bath. The SBF was refreshed every 2 days to preserve its ion concentration. After being soaked for selected durations, the specimens were removed from the fluid, washed with distilled water and air-dried.

After the NaOH treatment and ethyl alcohol aging, the surfaces of Ti-7.5Mo specimens were examined by field-emission scanning electron microscopy (FE-SEM; JSM-6700F, JEOL, Japan) and X-ray diffractometry (XRD; MXP-III, Brukers, Germany). Changes in the surfaces of Ti-7.5Mo after a period of soaking in SBF were determined by scanning electron microscopy (SEM; S-3000N, Hitachi, Japan), and surface chemical analysis was undertaken by energy-dispersive X-ray spectroscopy (EDS) in an 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

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

r	Na <sup>+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Cl <sup>-</sup>	HPO <sub>4</sub> <sup>2-</sup>	SO <sub>4</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>
Blood plasma	142.0	5.0	1.5	2.5	103.0	1.0	0.5	27.0
SBF	142.0	5.0	1.5	2.5	147.8	1.0	0.5	4.2

using an electrolyte-type pH meter (Ion 6 meter, Oakton, USA). The weight of the apatite coating on each specimen with a different soaking time was obtained in an electronic balance (SD-200L, Mirage, Japan) with an accuracy of  $\pm 0.0001$  g. The SBF was refreshed after each measurement. Contact angle measurements were implemented to evaluate the wettability of the chemically treated Ti-7.5Mo specimens. An equal volume of distilled water was placed on each sample by means of a micropipette, and it either formed a drop or spread across the surface of the sample. A charged-coupled device (CCD) camera was used to photograph the shape of the drops and measure the contact angle. Six specimens were used to evaluate the contact angles for each experimental condition.

### 3. Results and discussion

#### 3.1 Surface morphology after NaOH treatment and ethyl alcohol aging

Fig. 1 shows the changes in the surfaces of the NaOH-treated Ti-7.5Mo substrates after undergoing ethyl alcohol aging at 60 °C for 5 or 10 min. After the NaOH treatments, the surfaces of Ti-7.5Mo substrates exhibited porous network structures. These structures were relatively unchanged after various periods of ethyl alcohol aging, but gradually became denser after immersion in ethyl alcohol at 60 °C for 5 or 10 min. Uchida et al. (2002) also observed that the porous network structure of c.p. Ti, which was formed by the NaOH treatment, was apparently unchanged after immersion in water at 40 °C for 48 h, but gradually densified after immersion in water at 80 °C for 24 and 48 h.

Fig. 2 shows the XRD patterns of the surfaces of the Ti-7.5Mo substrates that were subjected to ethyl alcohol aging at 60 °C for 5 or 10 min after they were soaked in the NaOH solution. Previous studies have reported that this porous layer is sodium hydrogen titanate ( $\text{Na}_x\text{H}_{2-x}\text{Ti}_3\text{O}_7$ ) formed

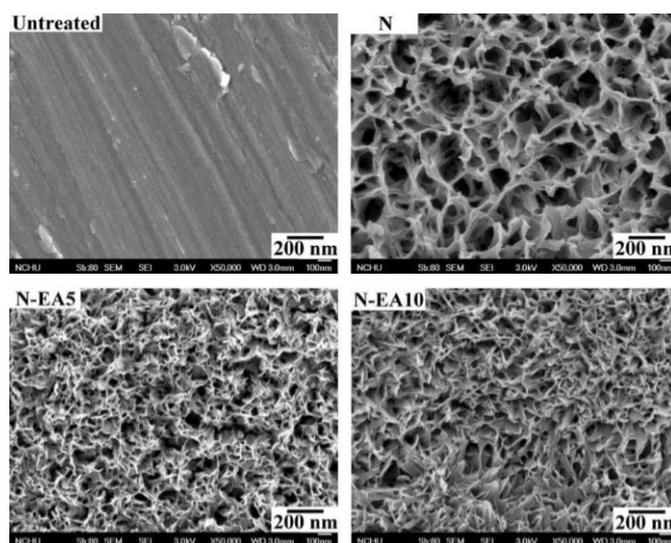


Fig. 1 FE-SEM photographs of Ti-7.5Mo surfaces subjected to NaOH (N) or NaOH-ethyl alcohol (N-EA) treatments for 5 or 10 min

after NaOH treatments (Yamaguchi *et al.* 2011 and Kawai *et al.* 2010). When the specimens were aged in ethyl alcohol, the sodium ions in the sodium hydrogen titanate hydrogel were released, a process similar to that which takes place when the specimens are soaked in SBF (Uchida *et al.* 2002 and Sun *et al.* 2008). Pattanayak *et al.* (2009) concluded that 5.5 at.% of Na was incorporated into the surface of c.p. Ti by the 5 M NaOH treatment, then completely removed by subsequent water or HCl treatments at 40 °C for 24 h. Uchida *et al.* (2002) also proposed that almost all the Na ions in the sodium titanate on the metal were released by the water treatments within 48 h at 40 °C, and within 24 h at 80 °C. As described in the results of a study by Hsu *et al.* (2011), EDS analysis of Ti-7.5Mo specimens water-aged for 24 or 48 showed no detectable Na signal. Notably, Kawai *et al.* (2010) recently proposed that Ti metal can achieve a stable and high apatite-forming ability when it is treated with water after the NaOH treatment to partially remove sodium ions in the surface layer, and then subjected to heat treatment.

### 3.2 Wettability

In the present study, NaOH treatment and ethyl alcohol aging were employed to augment hydrophilicity, which generally characterizes enhanced bioactivity (Kokubo *et al.* 2003). Fig. 3 shows the average water-contact angles of the untreated Ti-7.5Mo 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 27 ° for Ti-7.5Mo, after subsequent NaOH treatments the surface became more easily wetted, thus resulting in a very low contact angle (14 °). Moreover, the water-contact angles of the surfaces were further reduced by aging in ethyl alcohol. In fact, the contact angle of the Ti-7.5Mo sample which was ethyl alcohol-aged for 5 min was the lowest, being less than 9 °. Rupp *et al.* (2006) also found that hydrophobic sandblasted/acid-etched c.p. Ti surfaces could be induced into hydrophilic modification through 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 Ti-7.5Mo 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, and

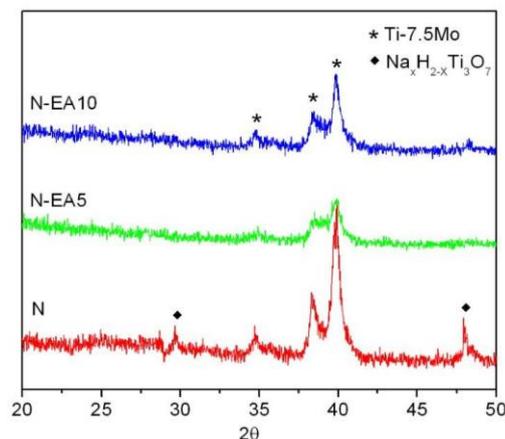


Fig. 2 XRD patterns of Ti-7.5Mo surfaces subjected to NaOH (N) or NaOH-ethyl alcohol (N-EA) treatments for 5 or 10 min

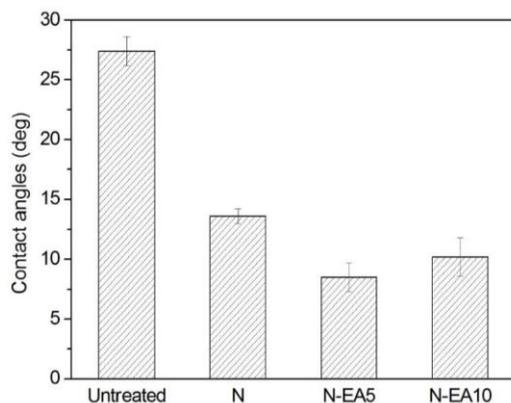


Fig. 3 Average water-contact angles of Ti-7.5Mo surfaces subjected to NaOH (N) or NaOH-ethyl alcohol (N-EA) treatments for 5 or 10 min

blood coagulation as well as cell and bacterial adhesion (Choe *et al.* 2004 and Faucheux *et al.* 2004). Highly hydrophilic surfaces are seen to be more desirable than hydrophobic ones in view of their interactions with biological fluids, cells and tissues (Zhao *et al.* 2005 and Buser *et al.* 2004). In an animal study, Buser *et al.* (2004) found that a hydrophilic sandblasted/acid-etched surface promoted enhanced bone apposition during the early stages of bone regeneration.

### 3.3 Ca-P precipitation on treated surfaces

Fig. 4 shows the SEM photographs of the surfaces of the Ti-7.5Mo specimens that had been soaked in SBF for 3, 7 or 14 days after NaOH treatment and had subsequently undergone different periods of aging in ethyl alcohol. As can be seen, the surfaces of Ti-7.5Mo that were not aged in ethyl alcohol were completely covered with a thin layer of calcium phosphate, and no obvious particle formation by nucleation was observed on the surfaces of the specimens that were soaked in SBF solution for 3 days. However, deposition and numerous nucleation spheres were noted on the surfaces of the ethyl alcohol-aged Ti-7.5Mo substrates within 3 days after immersion in SBF. Many more isolated or aggregated spheroids having a diameter of approximately 1–20  $\mu\text{m}$  were deposited on the surfaces of the Ti-7.5Mo treated with NaOH and subsequently aged in ethyl alcohol for 5 or 10 min. After soaking in SBF solution for 14 days, a dense calcium phosphate layer covered all the surfaces of the Ti-7.5Mo substrates, especially those specimens aged in ethyl alcohol, depleting the  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  from the surrounding solutions. This indicates that calcium phosphate can be quickly nucleated and deposited on the surface of Ti-7.5Mo after NaOH treatment and subsequent ethyl alcohol aging.

The results of the EDS analysis of the NaOH-treated and ethyl alcohol-aged surfaces after they were 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. Moreover, the intensities of the Ti peaks were much lower for the Ti-7.5Mo subjected to ethyl alcohol aging for 5 min. This result could be due to the interference from the calcium phosphate deposits which appeared after soaking, thereby indicating that these coatings were thick enough to prevent the penetration of X-ray beams into the substrate surface.

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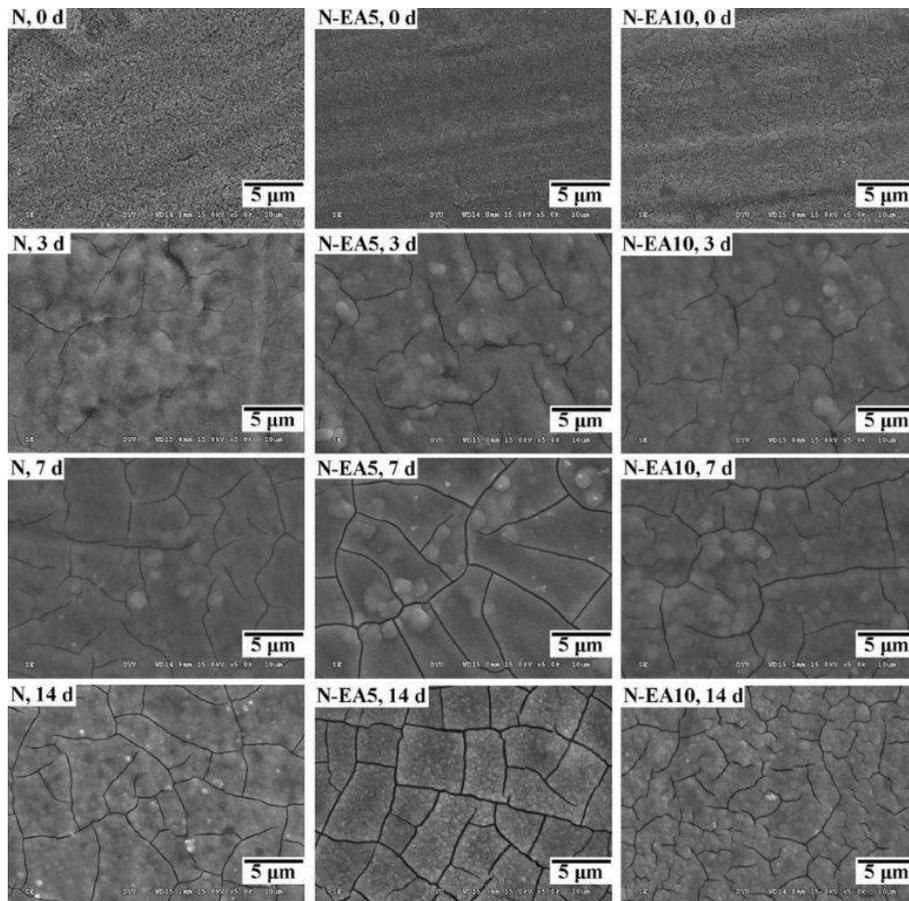


Fig. 4 SEM micrographs of surface morphology of NaOH-treated (N) and subsequently ethyl alcohol-aged (N-EA) Ti-7.5Mo after soaking in SBF for 3, 7 and 14 days

Throughout the period of immersion in SBF, the Ti-7.5Mo 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. In fact, the calcium phosphate deposits were significantly greater for the ethyl alcohol-aged Ti-7.5Mo 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 phosphates were much greater for the Ti-7.5Mo after ethyl alcohol aging for 5 min than for all other conditions. These results are also consistent with the observations recorded elsewhere in this report, as indicated in the results from SEM (Fig. 4) and EDS (Fig. 5).

The pH value of the SBF was found to increase gradually during the immersion of the NaOH-treated and subsequently ethyl alcohol-aged Ti-7.5Mo samples, thus indicating an increasing concentration of hydroxyl group (OH<sup>-</sup>) ions. This pH approached peaks of about 8.41–8.45 on the second day for the Ti-7.5Mo samples that received the NaOH and ethyl alcohol treatment. However, for samples that were not ethyl alcohol-aged, the pH approached peaks of 8.38 on the third day, as shown in Fig. 7. This pH increase in the SBF occurs during dissolution

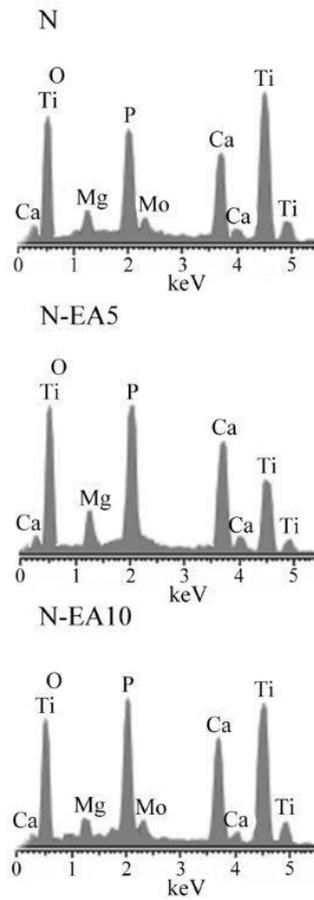


Fig. 5 EDS of NaOH-treated (N) and subsequently ethyl alcohol-aged (N-EA) Ti-7.5Mo after soaking in SBF for 14 days

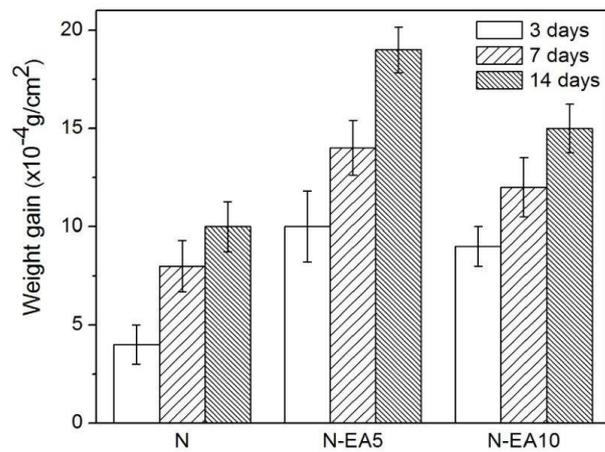


Fig. 6 Weight gains of NaOH-treated (N) and subsequently ethyl alcohol-aged (N-EA) Ti-7.5Mo after soaking in SBF for 3, 7 and 14 days

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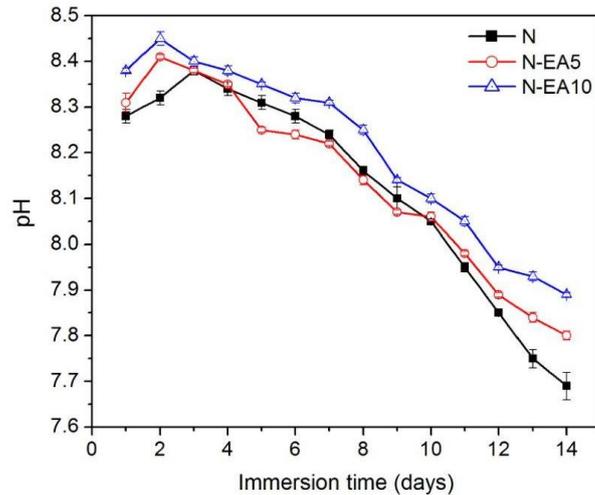


Fig. 7 pH values of SBF as functions of immersion time for Ti-7.5Mo subjected to NaOH (N) or NaOH-ethyl alcohol treatments (N-EA) for 5 or 10 min

due to the release of cations from the sodium titanate layer via an ion-exchange mechanism with protons ( $H^+/H_3O^+$ ) in the solution (Hench *et al.* 1991). During immersion in SBF, the pH values reached their maximum after either two or three days, and subsequently decreased with further soaking, thus implying that a continuous precipitation of the bone-like apatite layer would occur. In the present experiment, the calcium phosphate began to deposit on pre-treated substrates within 3 days of immersion in the SBF, which corresponds with the peak pH value attained on the second or third day.

It can be concluded that greater apatite-forming ability can be achieved by NaOH treatment and subsequent ethyl alcohol aging, a 5 min period of aging being ideal. After intensive investigations into the mechanisms of calcium phosphate 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 the SBF (Takadama *et al.* 2001 and Kokubo *et al.* 1996). After the NaOH-treated and ethyl alcohol-aged Ti-7.5Mo specimens are immersed in the SBF, the abundant Ti-OH groups are negatively charged (Kokubo *et al.* 2003, Kosmulski 1993 and Liu *et al.* 2004) and can combine with the positively charged  $Ca^{2+}$  ions that are in the SBF. If there are enough Ti-OH groups on the surface of the Ti metal, the  $Ca^{2+}$  ions can continually accumulate on the surface with the result that it gradually gains an overall positive charge. The positively charged surface combines with negatively charged phosphate ions to form calcium phosphate. Thus, the calcium phosphate formed in this manner initially takes on an amorphous structure but subsequently transform into more stable crystalline apatites (Kokubo *et al.* 2003 and Liu *et al.* 2004).

According to the present results, while all samples of Ti-7.5Mo subjected to NaOH treatment and ethyl alcohol aging show a greater calcium phosphate-forming ability than those without ethyl alcohol aging, after 10 min of ethyl alcohol treatment samples did not show a further increase in calcium phosphate-forming ability. This finding is also in agreement with the water-contact angles measured in this study (Fig. 5), which reached their lowest values after undergoing ethyl alcohol aging for 5 min. This is further confirmed by Wang *et al.* (2008) who found that enrichment of Ti-OH groups increases the surface energy (by decreasing the contact angle) on the Ti surfaces.

#### 4. Conclusions

In the present work, Ti-7.5Mo specimens were treated with NaOH, aged in ethyl alcohol and soaked in SBF to assess the calcium phosphate-formation abilities. The effects of various ethyl alcohol-aging times on the calcium phosphate-forming ability of Ti-7.5Mo were also evaluated. The results obtained in this research may be summarized as follows:

- The surface of the NaOH-treated Ti-7.5Mo substrates exhibited porous network structure. This structure became much denser after subsequently undergoing ethyl alcohol aging. Sodium hydrogen titanate ( $\text{Na}_x\text{H}_{2-x}\text{Ti}_3\text{O}_7$ ) reaction layers appeared on all Ti-7.5Mo samples after NaOH treatment.
- NaOH treatment of Ti-7.5Mo enhanced its surface wettability, and the water-contact angles of the surfaces were further reduced as the Ti-7.5Mo substrates were subsequently aged in ethyl alcohol. The contact angles measured after ethyl alcohol aging for 5 min were the lowest, being less than  $9^\circ$ .
- The surfaces of Ti-7.5Mo specimens that were not aged in ethyl alcohol were completely covered with a thin layer of calcium phosphate, and no particles formed on the surfaces of the specimens that were soaked in SBF solution for 3 days. However, there were numerous nucleation spheres and deposition on the surfaces of the ethyl alcohol-aged Ti-7.5Mo substrates within 3 days of immersion in SBF. After soaking in a SBF solution for 14 days, a dense calcium phosphate layer covered all the surfaces of the Ti-7.5Mo, particularly the ethyl alcohol-aged specimens.
- According to the results of the EDS analysis, the variation of pH in SBF, and the rates of weight gain, it can be concluded that Ti-7.5Mo substrates can achieve a greater apatite-forming ability through NaOH treatment and subsequent ethyl alcohol aging, a 5 min period of aging being ideal.

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#### References

- Ahmed, T., Long, M., Silvestri, J., Ruiz, C. and Rack, H.J. (1995), "A new low modulus, biocompatible titanium alloy", *Titanium 95: Sci. Technol.*, **2**, 1760-1767.
- Albayrak, O., El-Atwani, O. and Altintas, S. (2008), "Hydroxyapatite coating on titanium substrate by electrophoretic deposition method: effects of titanium dioxide inner layer on adhesion strength and hydroxyapatite decomposition", *Surf. Coat. Technol.*, **202**(11), 2482-2487.
- Barrère, F., Van der Valk, C.M., Meijer, G., Dalmeijer, R.A.J., De Groot, K. and Layrolle, P. (2003), "Osteointegration of biomimetic apatite coating applied onto dense and porous metal implants in femurs of goats", *J. Biomed. Mater. Res. B. Appl. Biomater.*, **67**(1), 655-665.
- Boyd, A.R., Duffy, H. and McCann, R. (2008), "Sputter deposition of calcium phosphate/titanium dioxide hybrid thin films", *Mater. Sci. Eng. C*, **28**(2), 228-236.
- Buser, D., Broggin, N., Wieland, M., Schenk, R.K., Denzer, A.J., Cochran, D.L., Hoffmann, B., Lussi, A. and Steinemann, S.G. (2004), "Enhanced bone apposition to a chemically modified SLA titanium surface", *J. Dent. Res.*, **83**(7), 529-533.

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- Chen, Y., Zheng, X., Ji, H. and Ding, C. (2007), "Effect of Ti-OH formation on bioactivity of vacuum plasma sprayed titanium coating after chemical treatment", *Surf. Coat. Technol.*, **202**(3), 494-498.
- Choe, J.H., Lee, S.J., Lee, Y.M., Rhee, J.M., Lee, H.B. and Khang, G. (2004), "Proliferation rate of fibroblast cells on polyethylene surfaces with wettability gradient", *J. Appl. Polym. Sci.*, **92**(1), 599-606.
- De Groot, K., Geesink, R. and Klein, C.P.A.T. (1987), "Serejian P. Plasma sprayed coatings of hydroxylapatite", *J. Biomed. Mater. Res.*, **21**(12), 1375-1381.
- Ducheyne, P., Radin, S., Heughebaert, M. and Heughebaert, J.C. (1990), "Calcium phosphate ceramic coatings on porous titanium: effect of structure and composition on electrophoretic deposition, vacuum sintering and in vitro dissolution", *Biomater.*, **11**(4), 244-254.
- Faucheux, N., Schweiss, R., Lutzow, K., Werner, C. and Groth, T. (2004), "Selfassembled monolayers with different terminating groups as model substrates for cell adhesion studies", *Biomater.*, **25**(14), 2721-2730.
- Faure, J., Balamurugan, A., Benhayoune, H., Torres, P., Balossier, G. and Ferreira, J.M.F. (2009), "Morphological and chemical characterisation of biomimetic bone like apatite formation on alkali treated Ti6Al4V titanium alloy", *Mater. Sci. Eng., C*, **29**(4), 1252-1257.
- Geesink, R.G. and Hoefnagels, N.H. (1995), "Six-year results of hydroxyapatite-coated total hip replacement", *J. Bone Joint Surg. Br.*, **77b**(4), 534-547.
- Hench, L.L. (1991), "Bioceramics: From concept to clinic", *J. Am. Ceram. Soc.*, **74**(7), 1487-1510.
- Ho, W.F. (2008a), "A comparison of tensile properties and corrosion behavior of cast Ti-7.5Mo with c.p. Ti, Ti-15Mo and Ti-6Al-4V alloys", *J. Alloys Compd.*, **464**(1-2), 580-583.
- Ho, W.F. (2008b), "Effect of omega phase on mechanical properties of Ti-Mo alloys for biomedical application", *J. Med. Bio. Eng.*, **28**(1), 47-51.
- Ho, W.F., Ju, C.P. and Chern Lin, J.H. (1999), "Structure and properties of cast binary Ti-Mo alloys", *Biomater.*, **20**(22), 2115-2122.
- Ho, W.F., Lai, C.H., Hsu, H.C. and Wu, S.C. (2010), "Surface modification of a Ti-7.5Mo alloy using NaOH treatment and Bioglass® coating", *J. Mater. Sci. Mater. Med.*, **21**(5), 1479-1488.
- Hsu, H.C., Tsou, H.K., Hsu, S.K., Wu, S.C., Lai, C.H. and Ho, W.F. (2011), "Effect of water aging on the apatite formation of a low-modulus Ti-7.5Mo alloy treated with aqueous NaOH", *J. Mater. Sci.*, **46**(5), 1369-1379.
- Hsu, H.C., Wu, S.C., Fu, C.L. and Ho, W.F. (2010), "Formation of calcium phosphates on low-modulus Ti-7.5Mo alloy by acid and alkali treatments", *J. Mater. Sci.*, **45**(13), 3661-3670.
- Im, K.H., Lee, S.B., Kim, K.M. and Lee, Y.K. (2007), "Improvement of bonding strength to titanium surface by sol-gel derived hybrid coating of hydroxyapatite and titania by sol-gel process", *Surf. Coat. Technol.*, **202**(4-7), 1135-1138.
- Kawai, T., Kizuki, T., Takadama, H., Matsushita, T., Unuma, H., Nakamura, T. and Kokubo, T. (2010), "Apatite formation on surface titanate layer with different Na content on Ti metal", *J. Ceram. Soc. Jpn.*, **118**(1373), 19-24.
- Kim, H.M., Miyaji, F., Kokubo, T. and Nakamura, T. (1996), "Preparation of bioactive Ti and its alloys via simple chemical surface treatment", *J. Biomed. Mater. Res.*, **32**(3), 409-417.
- Klein, C.P.A.T., Patka, P., Van der Lubbe, H.B.M., Wolke, J.G.C. and De Groot, K. (1991), "Plasma-sprayed coatings of tetracalciumphosphate, hydroxyl-apatite, and  $\alpha$ -TCP on titanium alloy: an interface study", *J. Biomed. Mater. Res.*, **25**(1), 53-65.
- Kokubo, T. (1996), "Formation of biologically active bone-like apatite on metals and polymers by a biomimetic process", *Thermochim. Acta.*, **280-281**, 479-490.
- Kokubo, T. and Kim, H.M. (2003), "Kawashita M. Novel bioactive materials with different mechanical properties", *Biomater.*, **24**(13), 2161-2175.
- Kokubo, T. and Takadama, H. (2006), "How useful is SBF in predicting in vivo bone bioactivity", *Biomater.*, **27**(15), 2907-2915.
- Kokubo, T., Miyaji, F., Kim, H.M. and Nakamura, T. (1996), "Spontaneous formation of bone-like apatite layer on chemically treated titanium metals", *J. Am. Ceram. Soc.*, **79**(4), 1127-1129.
- Kosmulski, M. (1993), "The role of the activity coefficients of surface groups in the formation of surface charge of oxides. Part II: Ion exchange and  $\zeta$  potentials", *Colloid Polym. Sci.*, **271**(11), 1076-1082.

- Lautenschlager, E.P. and Monaghan, P. (1993), "Titanium and titanium alloys as dental materials", *Int. Dent. J.*, **43**(3), 245-253.
- Lin, D.J., Chuang, C.C., Chern Lin, J.H., Lee, J.W., Ju, C.P. and Yin, H.S. (2007), "Bone formation at the surface of low modulus Ti-7.5Mo implants in rabbit femur", *Biomater.*, **28**(16), 2582-2589.
- Liu, X.Y., Chu, P.K. and Ding, C.X. (2004), "Surface modification of titanium, titanium alloys, and related materials for biomedical applications", *Mater. Sci. & Eng. R.*, **47**(3-4), 49-121.
- Lu, X., Leng, Y., Zhang, X.D., Xu, J.R., Qin, L. and Chan, C.W. (2005), "Comparative study of osteoconduction on micromachined and alkali-treated titanium alloy surfaces in vitro and *in vivo*", *Biomater.*, **26**(4), 1793-1801.
- McNally, S.A., Shepperd, J.A., Mann, C.V. and Walczak, J.P. (2000), "The results at nine to twelve years of the use of a hydroxyapatite-coated femoral stem", *J. Bone. Joint. Surg. Br.*, **82**(3), 378-382.
- Moroni, A., Toksvig-Larsen, S., Maltarello, M.C., Orienti, L., Stea, S. and Giannini, S. (1998), "A comparison of hydroxyapatite-coated, titanium-coated, and uncoated tapered external-fixation pins. an *in vivo* study in sheep", *J. Bone. Joint. Surg. Am.*, **80**(4), 547-554.
- Nishiguchi, S., Kato, H., Fujita, H., Kim, H.M., Miyaji, F., Kokubo, T. and Nakamura, T. (1999), "Enhancement of bone-bonding strengths of titanium alloy implants by alkali and heat treatments", *J. Biomed. Mater. Res.*, **48**(5), 689-696.
- Okazaki, Y., Rao, S., Tateishi, T. and Ito, Y. (1998), "Cytocompatibility of various metal and development of new titanium alloys for medical implants", *Mater. Sci. Eng. A.*, **243**(1-2), 250-256.
- Ong, J.L., Lucas, L.C., Lacefield, W.R. and Rigney, E.D. (1992), "Structure, solubility and bond strength of thin calcium phosphate coatings produced by ion beam sputter deposition", *Biomater.*, **13**(4), 249-254.
- Pattanayak, D.K., Kawai, T., Matsushita, T., Takadama, H., Nakamura, T. and Kokubo, T. (2009), "Effect of HCl concentrations on apatite-forming ability of NaOH-HCl- and heat-treated titanium metal", *J. Mater. Sci. Mater. Med.*, **20**(12), 2401-2411.
- Ratner, B.D. (2001), "Replacing and renewing: synthetic materials, biomimetics, and tissue engineering in implant dentistry", *J. Dent. Edu.*, **65**(12), 1340-1347.
- Rohanizadeh, R., Al-Sadeq, M. and LeGeros, R.Z. (2004), "Preparation of different forms of titanium oxide on titanium surface: Effects on apatite deposition", *J. Biomed. Mater. Res. A.*, **71**(2), 343-352.
- Rupp, F., Scheideler, L., Olshanska, N., De Wild, M. and Wieland, M. (2006), "Geis-Gerstorfer J. Enhancing surface free energy and hydrophilicity through chemical modification of microstructured titanium implant surfaces", *J. Biomed. Mater. Res. A.*, **76**(2), 323-334.
- Sun, T. and Wang, M. (2008), "Low-temperature biomimetic formation of apatite/TiO<sub>2</sub> composite coatings on Ti and NiTi shape memory alloy and their characterization", *Appl. Surf. Sci.*, **255**(2), 396-400.
- Takadama, H., Kim, H.M., Kokubo, T. and Nakamura, T. (2001), "TEM-EDX study of mechanism of bone-like apatite formation on bioactive titanium metal in simulated body fluid", *J. Biomed. Mater. Res.*, **57**(3), 441-448.
- Uchida, M., Kim, H.M., Kokubo, T., Fujibayashi, S. and Nakamura, T. (2002), "Effect of water treatment on the apatite-forming ability of NaOH-treated titanium metal", *J. Biomed. Mater. Res. B. Appl. Biomater.*, **63**(5), 522-530.
- Wang, X.J., Li, Y.C., Lin, J.G., Hodgson, P.D. and Wen, C.E. (2008), "Apatite-inducing ability of titanium oxide layer on titanium surface: the effect of surface energy", *J. Mater. Res.*, **23**(6), 1682-1688.
- Yamaguchi, S., Takadama, H., Matsushita, T., Nakamura, T. and Kokubo, T. (2011), "Preparation of bioactive Ti-15Zr-4Nb-4Ta alloy from HCl and heat treatments after an NaOH treatment", *J. Biomed. Mater. Res. A.*, **97**(2), 135-144.
- Yee, A.J., Kreder, H.K., Bookman, I. and Davey, J.R. (1999), "A randomised trial of hydroxyapatite coated prostheses in total hip arthroplasty", *Clin. Orthop. Relat. R.*, **366**, 120-132.
- Zhao, G., Schwartz, Z., Wieland, M., Rupp, F., Geis-Gerstorfer, J., Cochran, D.L. and Boyan, B.D. (2005), "High surface energy enhances cell response to titanium substrate microstructure", *J. Biomed. Mater. Res. A.*, **74**(1), 49-58.