

# Nanostructured Ti6Al4V alloy fabricated using modified alkali-heat treatment: Characterization and cell adhesion



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## ABSTRACT

In order to optimize the creation of a nanostructured surface on Ti6Al4V titanium alloy, an alkali treatment was performed using a 10-M NaOH solution at various temperatures (30, 40, 50, and 60 °C) so as to determine the optimal temperature. This was combined with subsequent heat treatments (200, 400, 600, and 800 °C) in air. The effects of different temperatures for the latter treatments on the nanostructure surface and the initial cell adhesion were evaluated, and the optimal temperature of the alkali solution was found to be 30 °C. Further, the nanotopography, surface chemistry, and surface roughness of the nanoporous structure were retained after heat treatments performed at 200, 400, and 600 °C, and only the phase structure was altered. The amorphous sodium titanate phase, the content of which increased with increased heat-treatment temperature, may have played a role in promoting cell adhesion on the nanoporous surface. However, heat treatment at 800 °C did not enhance the cell–surface attachment. Rather, the nanostructure degraded significantly with the reappearance of Al and V.

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## 1. Introduction

Titanium (Ti) and its alloys are widely used in the biomedical field. Titanium–aluminum–vanadium alloy (Ti6Al4V) has been used as implants due to its high biocompatibility and ability to allow bone-implant integration and excellent mechanical strength. If the application of Ti6Al4V is to be extended to dental implants, it is required to possess good corrosion resistance, excellent bioactivity, and freedom from toxic elements. Furthermore, dental implants must be subjected to surface pre-treatments if successful osseointegration is to be achieved. Therefore, many techniques have been employed to modify the surfaces of Ti and its alloys [1,2]. For example, Ti alloys coated with hydroxyapatite via a plasma spray method have been used in clinical applications. However, questions remain concerning their use; for example, despite the significant efforts that have been made to improve the abovementioned properties over a number of years, the adhesive strength of the coatings to the metallic substrates and their long-term reliability remain unclear [3,4].

Alkali-plus-heat (alkali-heat) treatments are among the effective surface bioactive treatments that have been applied to Ti and its alloys. Alkali-heat treatment results in the spontaneous formation of a bone-like apatite layer on the macroporous Ti surface in simulated body fluid (SBF), and bonding to bone occurs in vivo via this apatite layer. This simple chemical treatment has shown considerable promise as

regards hip arthroplasties [5]. Recently, other chemical treatments have been combined with the alkali-heat process to incorporate ions or nanoparticles (hydroxyapatite or calcium phosphate) into the Ti, thus inducing a specific biological response on the metallic surface [6–9].

In isolation, alkali-heat treatment is considered to be a basic surface modification technique that can provide an initial bioactive porous structure. Furthermore, subsequent heat treatment appears to be an essential aspect of alkali-heat-based techniques. Several in vivo studies have shown that alkali-heat-treated Ti and Ti6Al4V alloy can bond directly to bone with high bonding strength following subsequent heat treatment, whereas fibers attached to the implant surface have exhibited a lack of bone-bonding ability after a single alkali treatment [10–12]. However, the effect of heat treatment on the behavior of osteoblasts has been only minimally investigated.

Recently, we fabricated nanoporous structures on Ti metal and Ti6Al4V alloy using an alkali treatment method involving immersion in a 10-M NaOH solution at room temperature. The modification of the surface hydrophilicity and the nanotopography significantly enhanced the adhesion of the osteogenic cells and their differentiation [13–17]. Compared with conventional materials, nanostructured materials may have the ability to induce osseointegration more efficiently, by promoting specific protein interactions [18,19]. Low-cost, high-engineering-potential nanotechnological surface-modification treatments for dental implants have become a topic of considerable interest [20–24], and our improved alkali treatment shows potential as such a technique.

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The purpose of the present study is to further the development of bioactive treatments for the fabrication of nanoporous structures on Ti6Al4V alloy using a modified alkali-heat treatment. The alkali immersion temperature and the subsequent heat treatments were varied, and the influence of these changes on the surface characteristics of the resultant nanostructures and the osteogenic cell adhesion was analyzed.

## 2. Materials and methods

### 2.1. Specimen preparation

Ti6Al4V disks (chemical composition (wt.%): N: 0.02; C: 0.03; H: 0.011; Fe: 0.22; O: 0.16; Al: 6.12; V: 3.93; and Ti: the balance, 15 mm in diameter and 1 mm in thickness) were prepared as the substrate material (Daido Steel Co., Osaka, Japan). Nanoporous Ti6Al4V alloy was produced using a concentrated alkali solution at room temperature, as described in the literature [16]. The alloy disks were successively polished using several grades of SiC abrasive paper (600, 800, 1000, and 1500 grit), ultrasonically rinsed using acetone, ethanol, and distilled water (each for 10 min), and dried in air. The disks were immersed in a 10-M NaOH solution at 30, 40, 50, or 60 °C for 24 h, washed with distilled water, and dried at room temperature overnight so as to yield nanostructured alloy samples. The samples were first observed using scanning electron microscopy (SEM; S-4800, Hitachi Co., Tokyo, Japan), in order to determine the optimum NaOH immersion temperature. Following alkali treatment at 30 °C, the samples were placed in an electric furnace and heated to 200, 400, 600, or 800 °C using a heating rate of 5 °C/min under an air atmosphere. These samples were maintained at the desired temperature for 1 h, and then allowed to naturally cool to room temperature.

### 2.2. Surface analysis

Surface characterization of the alkali-heat-treated samples was performed using SEM with an accelerating voltage of 10 kV. Atomic force microscopy analyses (AFM; SPM-9600, Shimadzu Co., Tokyo, Japan) were also performed, in order to obtain the mean average surface roughness (Ra), mean peak-to-valley height (Rz), and two-dimensional surface topography. The surface chemical composition was investigated using X-ray photoelectron spectrometry (XPS; PHI X-tool, ULVAC-PHI, Inc., Kanagawa, Japan). The thickness of the modified layer on the Ti6Al4V was determined using XPS Ar-etching, by the product of the sputtering times of the relative elements (Ti, O, Na, Al, and V) and the etching rate [25]. The etching rate was approximately 4.32 nm/min (determined from standard instrument parameters). Finally, the surface phase properties were investigated using X-ray powder diffraction (XRD; XRD-6100, Shimadzu Co.). The spectra were recorded in the range of  $2\theta = 20^\circ$ – $60^\circ$ , operating at 40 kV and 200 mA and using a Cu-K $\alpha$  radiation source, with a scanning speed of 2°/min and an incident angle of 1°.

### 2.3. Cell culture

Rat Bone Marrow Mesenchymal Stem Cells (BMMSCs) were isolated and cultured according to our previously published procedures [14]. The rat BMMSCs were obtained from the femurs of 8-week-old Sprague Dawley rats. This experiment was conducted according to the Guidelines for Animal Experimentation of Osaka Dental University (approval No. 14-03,013). Primary cells were cultured at 37 °C in a 5% CO<sub>2</sub> atmosphere, in a growth medium containing minimal essential medium (Nacalai Tesque, Inc., Tokyo, Japan) supplemented with 10% fetal bovine serum (Nacalai Tesque, Inc.) and antibiotic–antimycotic mixed stock solution (Nacalai Tesque, Inc.). The culture medium was changed every 3 days.

### 2.4. Cell adhesion

The rat BMMSCs were seeded on the specimens at an initial density of  $4 \times 10^4$  cells/cm<sup>2</sup>, and the cell attachment was analyzed after 1, 3, 6, and 24 h. The nonadherent cells were removed via washing with phosphate-buffered saline (PBS) (Nacalai Tesque, Inc.) after each incubation interval. CellTiter-Blue Reagent (50  $\mu$ L) and PBS (250  $\mu$ L) were then added to each well. The cell numbers were measured using CellTiter-Blue Cell Viability Assay (Promega, Inc., Madison, WI, USA), in accordance with the manufacturer's protocol.

### 2.5. Statistical analyses

Statistical analyses were performed using a one-way analysis of variance (ANOVA), followed by Tukey's test. All of the results are shown as the mean  $\pm$  standard deviation (SD), calculated from five random areas in each sample. The differences were considered to be statistically significant for  $P < 0.05$ .

## 3. Results

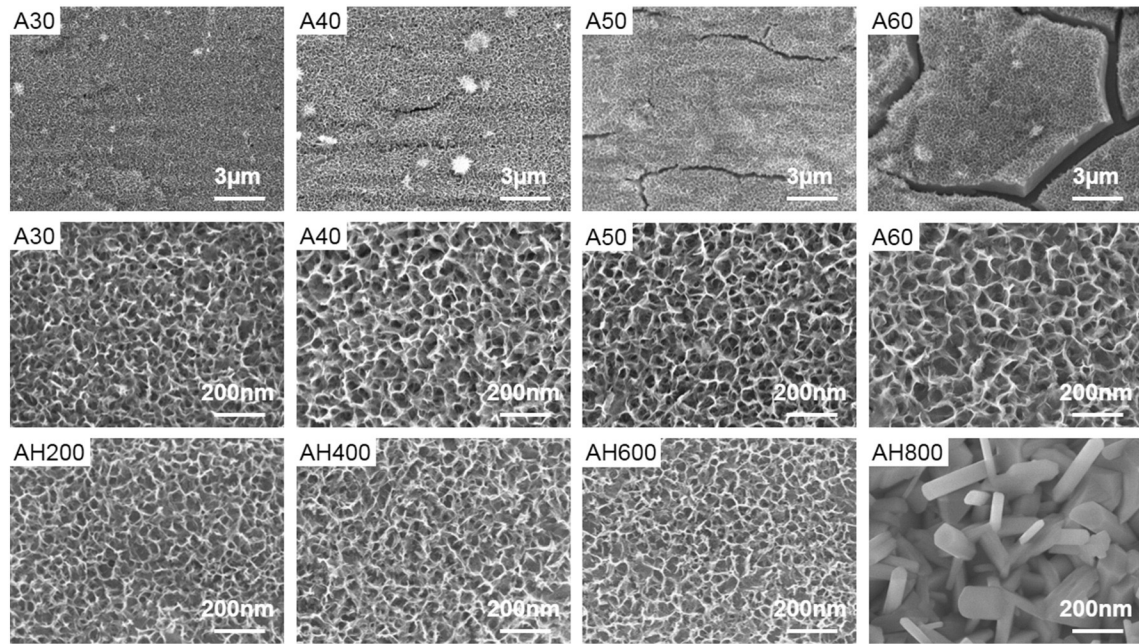
### 3.1. Surface morphology

The alkali treatment performed at different immersion temperatures using the 10-M NaOH solution yielded similar porous network structures on the Ti6Al4V alloy surfaces, as shown in the SEM images (Fig. 1). Irregular cracks were observed in the porous network structure for the alkali treatments performed at 40, 50, and 60 °C (A40, A50 and A60, respectively); however, no cracks were observed on the surface that was subjected to treatment at 30 °C (A30). SEM images recorded at higher magnification clearly showed that the fine pores were well interconnected, with an average diameter of approximately 50–100 nm after treatment at 30 °C. The pore size increased to 100–200 nm when the alkaline immersion temperature was increased. The alkali-heat-treated alloy specimens showed no significant variation in the nanoporous structures of the thermally oxidized Ti6Al4V alloy surfaces following the heat treatments performed at 200, 400, and 600 °C (AH200, AH400 and AH600, respectively); however, the nanoporous structure of the sample subjected to heat treatment at 800 °C (AH800) was damaged.

The AFM results confirmed that the nanostructured surfaces of the Ti6Al4V alloys subjected to heat treatments at 200–600 °C were retained (Fig. 2). Further, the nanoporous networks exhibited numerous sharp conical protrusions bordering the depression areas. After treatment at 800 °C, this morphology changed to a prismatic layer with crystals. The change in the surface roughness (Ra, Rz) was analyzed and the results are summarized in Table 1. There were no significant differences in the surface roughness characteristics of the samples before and after heat treatments performed at 200–600 °C (compared to the sample treated at an NaOH immersion temperature of 30 °C), but the roughness increased sharply after treatment at 800 °C.

### 3.2. Surface chemical analyses

Fig. 3 shows the results of the broad-range XPS surface chemical analyses of the Ti6Al4V alloys after they were subjected to alkali treatment in a 10-M NaOH solution at 30 °C, along with alkali-heat treatment at various subsequent heating temperatures. No significant differences are apparent in the XPS spectra of the specimens before and after the heat treatments performed at 200–600 °C, but Al and V peaks can be seen in the spectrum for the surface treated at 800 °C. The quantitative surface chemical compositions of the treated specimens are shown in Table 2. For the 200–600 °C cases, no V or Al were detected, and the relative atomic concentrations of the modified surfaces before (A30) and after the heat treatments are almost identical. However, the V and Al



**Fig. 1.** SEM photographs of specimens subjected to alkali treatments with 10-M NaOH solution at immersion temperatures of 30, 40, 50, and 60 °C (A30, A40, A50, and A60, respectively), and to alkali-heat treatments performed at heating temperatures of 200, 400, 600, and 800 °C (AH200, AH400, AH600, and AH800, respectively) following alkali treatment at 30 °C.

peaks reappeared when the temperature was increased to 800 °C, and the Al content exceeded the Ti content.

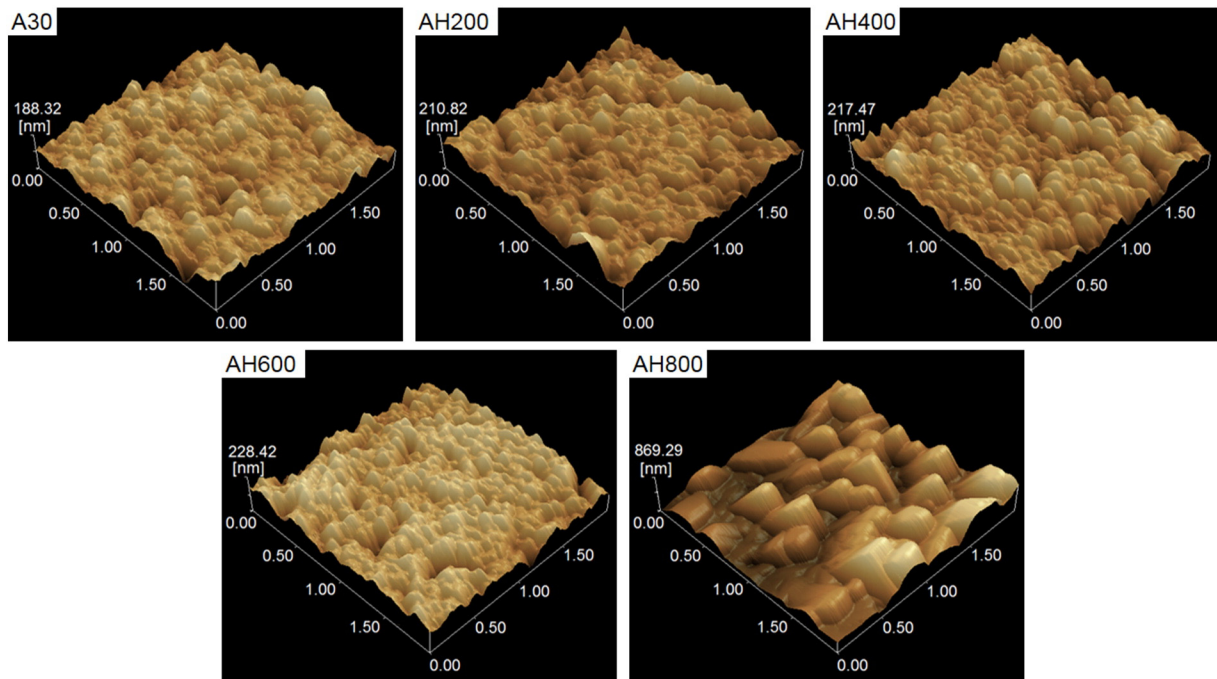
### 3.3. Depth-profiling study

XPS depth profiles of the NaOH-treated samples before and after the heat treatments are shown in Fig. 4. The thickness of the sodium titanate layer, which was free of the Al and V formed by the NaOH treatment, was determined to be approximately 216 nm. After heat treatment, the O moved toward deeper regions, but no significant variation was observed in the Na distribution. The oxide thickness of specimens

subjected to alkali-heat treatments performed at heating temperatures of 200, 400 and 600 °C were 721 nm, 756 nm and 982 nm, respectively, while alkali treated sample (A30) was 639 nm. The results showed that the Al and V content on the thermally oxidized surface was constant following the heat treatment performed at 800 °C.

### 3.4. XRD phase identification

The crystallographic structures of the modified surfaces were assessed before (alkali treatment at 30 °C) and after the heat treatments using XRD (Fig. 5), so as to determine the dependence of the phase



**Fig. 2.** AFM images of nanoscale surface features induced by alkali treatment at 30 °C (A30) and alkali-heat treatments with subsequent heating temperatures of 200, 400, 600, and 800 °C (AH200, AH400, AH600, and AH800, respectively).



**Table 1**

Surface roughness values of specimens subjected to alkali treatment at 30 °C (A30) and alkali-heat treatments (AH) under various subsequent heating temperatures.

	Ra (nm)	Rz (nm)
A30	19.890	188.535
AH200	20.653	210.717
AH400	21.903	217.261
AH600	22.476	228.324
AH800	165.384	972.140

transformation on the heat-treatment temperature. The broad diffraction peaks detected at approximately 23–29° and 53° were attributed to the presence of sodium hydrogen titanate, which is the main phase that forms the nanoporous network after the alkali treatment. When the heat-treatment temperature was increased from 200 to 400 °C, the intensity of these broad peaks increased as the sodium hydrogen titanate was gradually converted into amorphous sodium titanate. Complete transformation was achieved when the temperature was increased to 600 °C. Finally, transformation into a crystalline phase occurred after treatment at 800 °C. Sharp narrow peaks associated with rutile (TiO<sub>2</sub>) appeared in the sample following heat treatment at 800 °C. Further, a peak associated with alloy element oxide (Al<sub>2</sub>O<sub>3</sub>) was observed.

### 3.5. Cell adhesion assay

Fig. 6 shows the cell adhesion results for the first 24 h. The cell adhesion on the single-alkali-treated samples was significantly lower ( $P < 0.05$ ) than that observed in the case of the samples alkali-heat-treated at 200, 400, and 600 °C, for all culture durations. The number of adherent cells increased gradually when the heat-treatment temperature was increased in the 200–600 °C range; however, the number of adherent cells decreased in the sample subjected to heat treatment at 800 °C.

## 4. Discussion

A novel bioactive material with nanoporous features was obtained on Ti6Al4V alloy using a modified alkali-heat treatment. The initial alkali-treatment temperature and the subsequent heat treatments

**Table 2**

Surface chemical compositions of specimens subjected to alkali treatment (A30) and alkali-heat treatments (AH) under various subsequent heating temperatures.

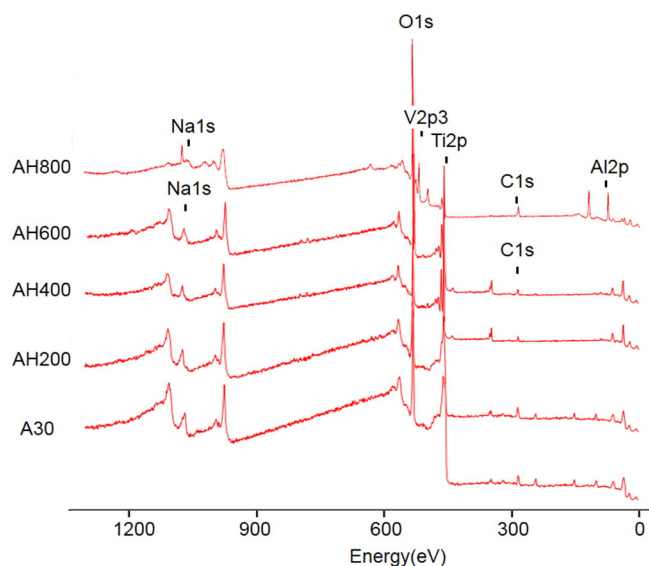
	Chemical composition (at.%)				
	O	Na	Al	Ti	V
A30	66.52	4.95	0	28.53	V
AH200	66.65	5.05	0	28.30	0
AH400	67.03	5.07	0	27.90	0
AH600	68.35	4.25	0	27.40	0
AH800	70.13	0.07	14.80	10.25	4.75

played a significant role in determining the surface characteristics of the nanostructure and the initial cell adhesion properties.

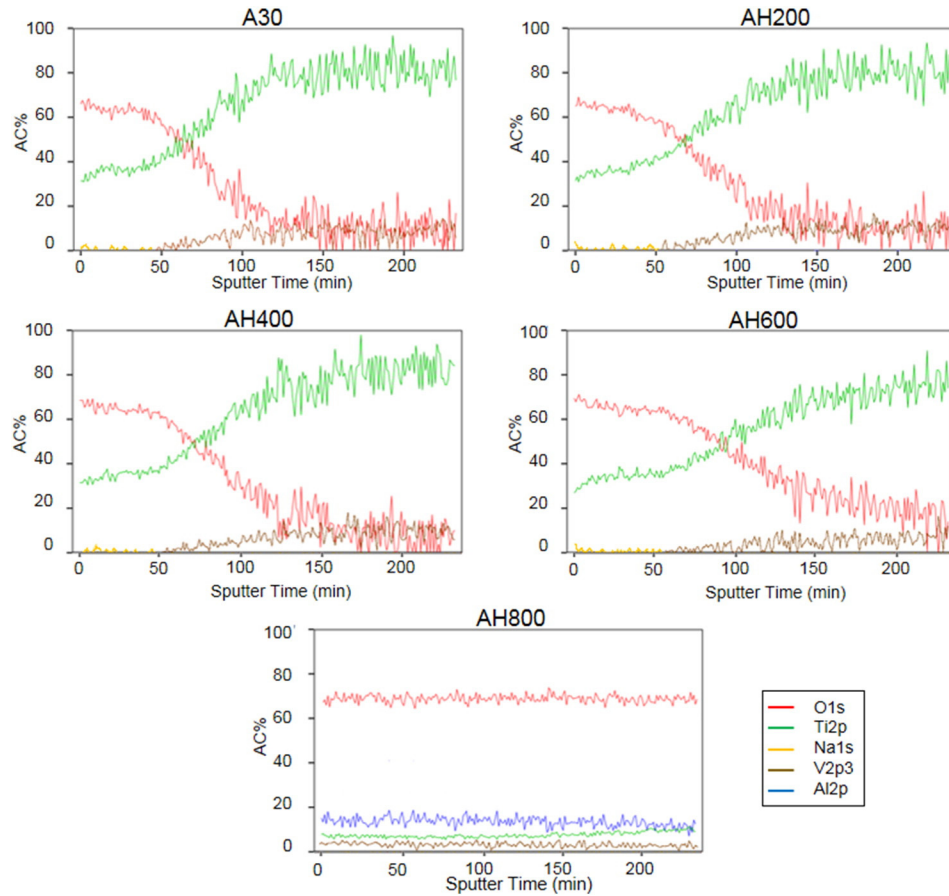
The surface characterization results showed that the 10-M NaOH solution alkali treatment performed at 30 °C produced a fine nanoporous network. It was proposed that the pore size of the porous network depended on the concentration and the temperature of the alkali solution. Conventional alkaline treatments performed using 5-M NaOH produce a surface morphology with a macroporous structure; in contrast, the method used in the current study produced a nanoporous structure. The mechanism responsible for the formation of porous networks on Ti metal or its alloys during alkali treatment involves a corrosive attack by the hydroxyl groups [26]. In this study, the formation of the nanostructured surface likely resulted from the use of the 10-M NaOH concentration during the alkali treatment. This higher concentration provided more hydroxyl groups than could be incorporated into the metal surface, and subsequently induced the formation of a higher number of pores. However, the pore size increased beyond the nanoscale level when the immersion temperature was increased from 30 to 60 °C. This suggests that a further hydroxyl attraction was created around the dissolved substrate region as a result of increases in the immersion temperature, leading to increased pore size. The number of irregular cracks in the porous network also increased when the immersion temperature was increased in the 40–60 °C range; this was because the sodium hydrogen titanate layer dehydrated during the drying process. These results indicate that the 10-M NaOH solution immersion temperature necessary to produce the desired nanostructured surface on the Ti6Al4V is 30 °C.

In this study, the depth analysis of the oxide layer (performed using XPS) showed that the thickness of the sodium hydrogen titanate layer was 216 nm, compared to the 1 µm yielded by conventional alkali treatments [27]. This indicates that, despite the high concentration of NaOH, the rate of the reaction between the Ti6Al4V substrate and the hydroxyl groups was slow because of the low alkaline treatment temperature of 30 °C. It was therefore expected that extending the duration of the alkaline treatment using our modified alkali-heat treatment would allow a thicker sodium hydrogen titanate layer to be produced.

Heat treatment is an important process as regards enhancing the thickness of the oxide layer on the Ti6Al4V surface after the initial NaOH treatment. A uniform oxygen diffusion layer (TiO<sub>x</sub>,  $0 < x \leq 2$ ) was shown to newly appear beneath the porous sodium titanate layer when the Ti substrate was subjected to heat treatment in air after alkali treatment, but this was not present in a sample heat treated in a vacuum in a previous study [28]. The signal intensity of the corresponding peak of the suboxide layer increased when the heat treatment temperature was increased to 600 °C, and was associated with rutile (TiO<sub>2</sub>) based on an XRD analysis (Fig. 5). Further, the thickness of the oxide layer increased following the heat treatments as a result of the formation of the TiO<sub>2</sub> layer between the porous sodium titanate layer and the alloy substrate. Note that the oxygen penetration depth into a substrate is dependent on the heat treatment and the atmosphere, and depths of approximately 500 nm to 1.5 µm for heat treatment in air and 200–500 nm for heat treatment in vacuum have been reported [29–31]. In this study, the thickness of the oxide layer increased when the heat-treatment temperature was increased in an air atmosphere



**Fig. 3.** XPS survey spectra of Ti6Al4V alloy specimens subjected to alkali treatment (A30) and alkali-heat treatments (AH) with subsequent heating at temperatures of 200, 400, 600, and 800 °C.



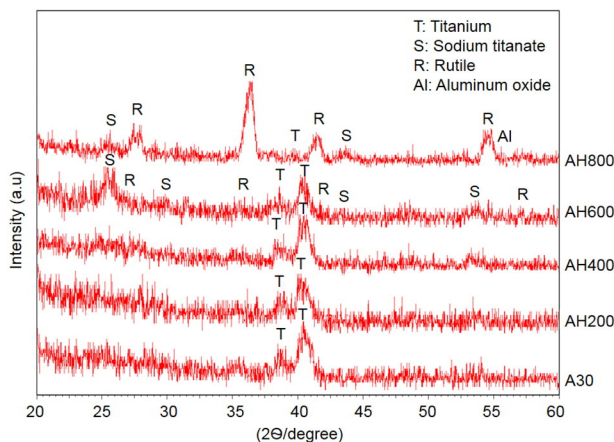
**Fig. 4.** Depth profiles of modified surfaces on Ti6Al4V specimens subjected to alkali treatment (A30) and alkali-heat treatments (AH) at various temperatures, obtained via XPS Ar-etching.

(Fig. 4). This suggests that the heat treatment temperatures have a significant effect on the oxygen diffusion into the substrate.

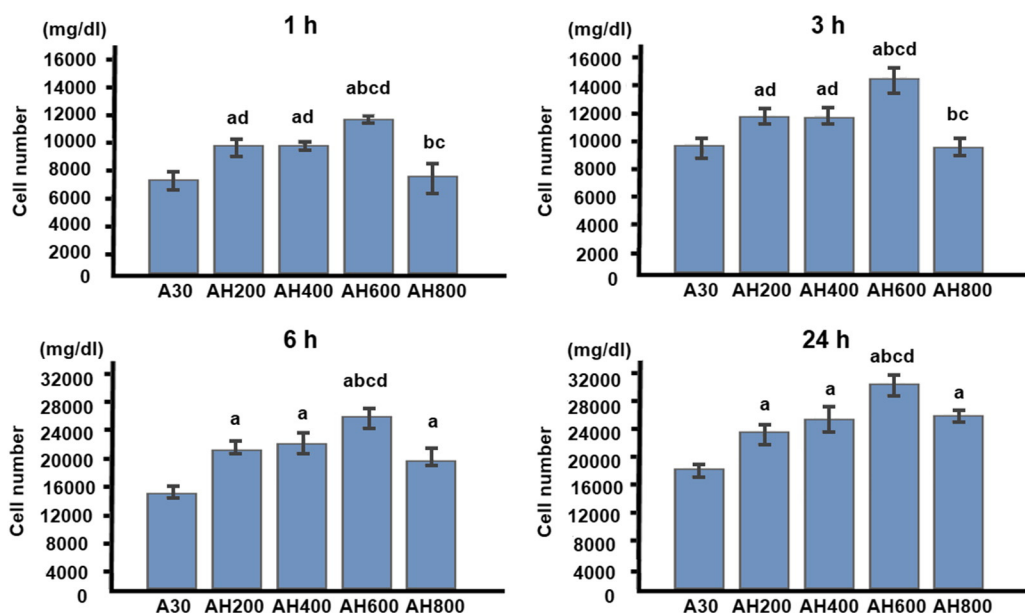
The biocompatibility of the Ti6Al4V alloy, the lack of which is the major obstacle to application of this alloy as a biomedical material, is related to the release of ions from the Ti6Al4V [32]. Our results confirmed that the surface chemical composition of the alkali-treated alloy without Al and V was identical before and after the heat treatments performed at temperatures of 200–600 °C. Previously, it has been demonstrated that the selective dissolution of the Al and V alloy species into the alkaline solution, along with stabilization of the Al and V in the TiO<sub>2</sub> layer after heat treatment, contribute to a reduction in the toxicity of the Ti6Al4V

alloy [33,34]. Further, Kobayashi has found that a graded surface consisting of a sodium titanate layer, a dense sodium titanate film, a TiO<sub>2</sub> layer, and the alloy substrate, is formed during heat treatment at 600 °C [35]. We believe that the existence of a dense sodium titanate film played a vital role in preventing the dissociation of Al and V from the inner substrate in this study. As regards the reappearance of Al and V in the sodium titanate layer at 800 °C, note that Takadama has reported that the binding energies of Al and V in their oxides decrease after heat treatment, which leads to the easy detection of Al and V at high temperatures [36]. The XPS and XRD data for alkali-heat treatment performed at heating temperature of 800 °C show that alloy elements were detected on the top of the modified surface with uniform coverage. Thus, it is likely that the multilayer structure of the graded surface was destroyed and that this translated into a crystal structure, resulting in a variation in the spatial distribution of the elements after the heat treatment at 800 °C. Therefore, the toxicity of the Ti6Al4V alloy could be negated through application of an alkali-heat treatment. However, it would be necessary to carefully control the heat treatment temperature in order to avoid damaging the graded structure.

It is recognized that the bioactive behavior of Ti metal and its alloys is induced by amorphous sodium titanate, the presence of which depends on the heating temperatures applied after the conventional alkaline treatment (5-M NaOH, 60 °C) [33,37]. Our XRD findings were in agreement with those results, which showed that the sodium hydrogen titanate was gradually transformed into amorphous sodium titanate and/or crystalline sodium titanate after heat treatment. It has been proposed that the appropriate heat-treatment temperature necessary to induce bioactivity in osteoblasts on thermally oxidized Ti6Al4V alloy is 500 or 700 °C [38]. However, Wei found that no apatite was deposited on a Ti6Al4V specimen in SBF that was immersed in a 5-M NaOH solution at 80 °C for 3 days and then subjected to heat treatment at



**Fig. 5.** TF-XRD patterns of surfaces subjected to alkali treatment (A30) and alkali-heat treatments (AH) with subsequent heating at temperatures of 200, 400, 600, and 800 °C.



**Fig. 6.** Initial number of adherent BMSCs on the surfaces of Ti6Al4V specimens subjected to alkali treatment (A30) and alkali-heat treatments (AH) with subsequent heating at temperatures of 200, 400, 600, and 800 °C. Statistical significance: (a) vs A30; (b) vs AH200; (c) vs AH400; (d) vs AH800.  $P < 0.05$ .

700 °C. This was because the sodium titanate layer was completely transformed into a crystal phase, which was too stable to exchange ions with the SBF [39]. The results of the present study clearly show that a heat-treatment temperature of 600 °C is the most favorable for the production of amorphous sodium titanate (Fig. 5). This is another fact to consider when determining the temperature to be applied in this alkali and heat treatment for clinical applications.

In the present study, cell adhesion of BMSCs was used to demonstrate the biocompatibility of the modified surface on the Ti6Al4V alloy. This adhesion is considered to be a critical prerequisite for cell proliferation and differentiation. A previous study has shown that the initial adhesion force of osteoblasts is improved on a Ti6Al4V alloy subjected to a high-temperature surface treatment [40]. Further, the differentiation behavior of osteoblasts on the surface of an alkali-heat-treated titanium-8tantalum-3niobium alloy has been found to be accelerated compared with that of a sample that is not subjected to heat treatment [41]. Cell attachment is a complicated process involving several surface features, including the topographic morphology, surface roughness, surface chemistry, and phase state. Here, we demonstrated the importance of the surface phase for cell adhesion, because the evidence provided in this study shows that there were no significant changes in the nanostructures, roughness, and chemical compositions of the samples before and after the heat treatments performed at temperatures of 200–600 °C. Instead, the amorphous sodium titanate content was the key factor influencing the enhancement of the cell adhesion. Therefore, this study is the first to provide evidence that the adhesion of BMSCs on a nanoporous structure with amorphous sodium titanate phases depends on the temperature used in the subsequent heat treatment.

In addition to the transformation of the crystal sodium titanate and the appearance of toxic elements (Al and V) that occurred when the temperature was increased to 800 °C, the distortion of the nanoporous structure and the changes in the nanoscale surface roughness at this temperature also had an adverse effect on the cell adhesion. The cell adhesion is sensitive to the material nanotopography, which imitates cellular environments so as to favor the process of rapid bone accrual. In cell adhesion to basement membranes, for example, the epithelial basement membrane structure contains pores of approximately 70–100 nm in size [42]. In this study, pore sizes of 50–100 nm were obtained in the nanoporous structures produced using the modified alkali-heat treatments (performed at 200–600 °C heating temperature); this pore-size range is similar to that of the basement membranes. The nanoscale

surface roughness also remained at approximately  $R_a = 20$  nm, even after the heat treatments (200–600 °C); this value is within the optimum surface roughness range of  $R_a = 10$ –45 nm, which has been shown to promote four types of cell adhesion in a previous report [43]. Further studies are necessary in order to investigate the proliferation and differentiation of osteoblasts on Ti6Al4V surfaces with nanoporous structures produced using modified alkali-heat treatments. Such studies will be helpful in achieving an improved understanding of the effects of heat treatment on the bioactivity of this novel material.

## 5. Conclusion

It was found that nanoporous structures were formed on the surfaces of Ti6Al4V alloys treated at 30 °C in 10-M NaOH aqueous solutions for 24 h. The formation of the nanostructured surfaces was ascribed to corrosive attacks due to the large amount of hydroxyl groups generated by the concentrated alkaline solution. Note that surface cracks developed on the specimens and the pore sizes increased beyond the nanoscale level when the alkaline solution immersion process was performed at 40, 50, or 60 °C. Following immersion in NaOH at 30 °C, test specimens were subjected to heat treatments at 200, 400, 600, or 800 °C in order to investigate the effect of temperature on the surface characteristics of the nanostructured surface and the cell adhesion of BMSCs. Surface characterization studies revealed that the nanotopography, surface chemistry, and surface roughness features of the modified layer obtained on each sample as a result of the alkali treatment were maintained even after heat treatments at 200, 400, or 600 °C. Only the phase structure was altered, particularly in the case of the amorphous sodium titanate phase, which may play a crucial role in promoting cell adhesion on the nanoporous surface with increasing temperature. This activity is thought to be more effective than that of the sodium hydrogen titanate obtained after a single alkali treatment. Further, the heat treatment conducted at 800 °C transformed the nanoporous structure into a macro-scale crystal structure and led to the reappearance of Al and V; hence, decreased cell attachment was induced. These results show that the nanoporous nature of the Ti6Al4V surface and the adhesion of BMSCs on this surface can be controlled and improved by varying the alkali immersion temperature and the temperature of the subsequent heat treatment. We found that the optimum results are obtained through immersion in a 10-M NaOH solution at 30 °C, along with heat treatment at 600 °C for 1 h. This modified



alkali-heat treatment technique has the potential to improve biological activity at cell–material interfaces.

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### Conference articles:

1. Yingmin Su et al., The effect of alkaline-treating temperature on nanostructure of Ti6Al4V, *Journal of the Japan Prosthodontic Society* (2014), ISSN: 1883–4426, p. 230.

2. Yingmin Su et al., Characterization and bioactivity of nanostructures fabricated on Ti-6Al-4 V alloy, 93rd General Session & Exhibition of the IADR, 2015/3/11–14.