XPS study of the process of apatite formation on bioactive Ti–6Al–4V alloy in simulated body fluid

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Abstract

Bioactive Ti–6Al–4V alloy, which spontaneously forms a bonelike apatite layer on its surface in the body and bonds to living bone through this apatite layer, can be prepared by producing an amorphous sodium titanate on its surface by NaOH and heat treatments. In this study, the process of apatite formation on the bioactive Ti–6Al–4V alloy was investigated in vitro, by analyzing its surface with X-ray photoelectron spectroscopy as a function of soaking time in a simulated body fluid (SBF). Thin-film X-ray diffractometry of the alloy surface and atomic emission spectroscopy of the fluid were also performed complementarily. It was found that immediately after immersion in the SBF, the alloy exchanged Na\textsuperscript{+} ions from the surface sodium titanate with H\textsubscript{2}O\textsuperscript{+} ions in the fluid to form Ti-OH groups on its surface. The Ti-OH groups, immediately after their formation, incorporated the calcium ions in the fluid to form calcium titanate. The calcium titanate thereafter incorporated the phosphate ions in the fluid to form an amorphous calcium phosphate, which was later crystallized into bonelike apatite. This process of apatite formation on the alloy was the same as on the pure titanium metal, because the alloy formed the sodium titanate free of Al and V by the NaOH and heat treatments. The initial formation of the calcium titanate is proposed to be a consequence of the electrostatic interaction of negatively charged units of titanite dissociated from the Ti-OH groups with the positively charged calcium ions in the fluid. The calcium titanate is postulated to gain a positive charge and interact with the negatively charged phosphate ions in the fluid to form amorphous calcium phosphate, which eventually stabilizes into crystalline apatite. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Ti–6Al–4V alloy; NaOH and heat treatments; Bioactivity; Apatite; Simulated body fluid; X-ray photoelectron spectroscopy

1. Introduction

Bioactive ceramics such as Bioglass® [1], sintered hydroxyapatite (HA) [2] and Cerabone® A/W [3], which spontaneously bond to living bone, are now widely available for bone-repairing materials. They bond to and integrate with living bone through a biologically active bonelike apatite layer which is formed on their surfaces in the body [4–7]. None of these bioactive ceramics, however, show fracture toughness as high as that of human cortical bone, thereby being unable to be used as substitutes for highly loaded bones such as femur and tibia. Materials currently available for this purpose are biocompatible metals with high fracture toughness, represented by titanium and its alloy [8]. None of these metals, however, bonds to bone, and therefore their stable fixations to the surrounding bone have been long considered as a fundamental problem in clinical uses.

Recently, the present authors showed that titanium metal can be highly bioactive by being simply soaked in an NaOH aqueous solution and subjected to a heat treatment [9–14]. Actually, the titanium treated in this way was found in vivo to bond to and integrate with living bone by forming a bonelike apatite layer on its surface in the body [15–18], as the bioceramics do. This bioactive titanium metal is now projected to clinical applications as load-bearing bone substitutes such as hip joints and dental roots.

The mechanism of apatite formation on its surface is a subject of much scientific interest, as it may provide fundamental understanding in bioactive surface functionalization of non-bioactive materials. Recent in vitro studies using a simulated body fluid (SBF), which is able to reproduce the in vivo bonelike apatite formation on bioactive materials [19–22], showed that the apatite formation on the bioactive titanium metal is due to an amorphous sodium titanate formed on the metal by NaOH and heat treatments [9,10,12,14]. The sodium titanate on the metal was shown to exchange its Na\textsuperscript{+} ions with H\textsubscript{2}O\textsuperscript{+} ions in the fluid to form Ti-OH groups effective for apatite nucleation [9–14,23,24]. The Ti-OH groups thereafter induce apatite nucleation.
Table 1
Ionic concentrations and pH of simulated body fluid in comparison with those in human blood plasma

| Ion     | Concentration (mM) | Simulated fluid | Blood plasma |
|---------|-------------------|-----------------|--------------|
| Na⁺     | 142.0             | 142.0           |              |
| K⁺      | 5.0               | 5.0             |              |
| Mg²⁺    | 1.5               | 1.5             |              |
| Ca²⁺    | 2.5               | 2.5             |              |
| Cl⁻     | 148.8             | 103.8           |              |
| HCO₃⁻   | 4.2               | 27.0            |              |
| HPO₄²⁻  | 1.0               | 1.0             |              |
| SO₄²⁻   | 0.5               | 0.5             |              |
| pH      | 7.40              | 7.20–40         |              |

indirectly, by forming an amorphous calcium titanate, which induces formation of an amorphous calcium phosphate as a precursor of the apatite [25,26]. The formation of calcium titanate was thereby proposed as the prerequisite for the apatite formation on the bioactive titanium metal.

The NaOH and heat treatments are effective for inducing apatite-forming ability even on titanium alloys such as Ti–6Al–4V [9,10], which are more often used as clinical bone substitutes than pure titanium metal [8]. In this context, it is a subject of importance whether the mechanism of apatite formation on bioactive titanium metal is consistently applicable on the titanium alloys after NaOH and heat treatments. In the present study, the process of apatite formation on bioactive Ti–6Al–4V alloy prepared by NaOH and heat treatments was investigated in vitro. The alloy surface was surveyed using X-ray photoelectron spectroscopy (XPS) as a function of soaking time in a SBF, which was complemented by thin-film X-ray diffractionmetry (TF-XRD) of the alloy surface and inductively coupled plasma atomic emission spectroscopy of the fluid. The mechanism of apatite formation was therefore discussed in terms of electrostatic interaction of the alloy surface with ion species in the SBF.

2. Materials and methods

2.1. Specimen preparation

Substrates of Ti–6Al–4V alloy (Kobe Steel Ltd, Kobe, Japan) 10 × 10 × 1 mm³ in size were abraded with No. 400 diamond plate, and ultrasonically cleaned in pure acetone and distilled water. Each of the substrate was soaked in 5.0 ml of 5.0 M NaOH aqueous solution at 60°C for 24 h, gently washed with distilled water, and dried at 40°C for 24 h. This NaOH treatment produces a bioactive sodium titanate gel layer on the alloy [11]. They were then placed in a Ni–Cr electric furnace, heated to 600°C at a rate of 5°C/min, kept at 600°C for 1 h, and then allowed to cool to room temperature in the furnace. This heat treatment is aimed to mechanically stabilize the bioactive gel layer into dehydrated amorphous sodium titanate [12].

2.2. Soaking of the specimen in simulated body fluid

Each of Ti–6Al–4V alloy substrates after the NaOH and heat treatments was soaked in 20 ml of an acellular SBF [19] at 36.5°C, whose pH and ionic concentrations are almost equal to those in human blood plasma as shown in Table 1. The SBF was prepared by dissolving reagent-grade NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂ and Na₂SO₄ (Nacalai Tesque Inc., Kyoto, Japan) into distilled water and buffering at pH 7.40 with tri-hydroxy-methyl aminomethane ((CH₂OH)₃CNH₂) and 1.0 M HCl (Nacalai Tesque Inc., Kyoto, Japan) at 36.5°C. After soaking for selected periods, the substrates were removed from the fluid, washed with distilled water, and dried on a clean bench.

2.3. Analyses of substrate and SBF

The surface of Ti–6Al–4V alloy substrate after the NaOH and heat treatments and after subsequent soaking in the SBF were analyzed by XPS (Model MT5500, ULVAC-PHI Co., Ltd, Chigasaki, Japan) and TF-XRD (Model 2651A1, Rigaku Co., Tokyo, Japan). In the XPS measurement, the photoelectron take-off angle was set at 45°, using MgKα (λ = 9.8903 Å) radiation as the source. The measured binding energies were corrected referring the energy position of C₁s in CH₂ (284.6 eV). The TF-XRD measurement was performed using CuKα (λ = 1.5405 Å) radiation as the source at a rate of 2° = 2°/min and 1° glancing angle against the incident beam on the alloy surface. As reference materials, untreated Ti–6Al–4V alloy, titania gel prepared by sol–gel method [27], calcium titanate (Ca₃Ti₂O₇) prepared by conventional powder pressing and sintering process, and sintered hydroxyapatite (HA Mitsubishi Mining Co., Kyoto, Japan) were subjected to the same XPS and TF-XRD analyses.

The element concentrations and pH of the SBF before and after soaking the alloy substrate were measured using inductively coupled plasma atomic emission spectroscopy (Model SPS1500, Seiko Inst. Inc., Chiba, Japan) and electrolyte-type pH meter (Model D-14, Horiba, Ltd, Kyoto, Japan), respectively.

3. Results

Fig. 1 shows the Ti₂p XPS spectra of the surface of the Ti–6Al–4V alloy subjected to the NaOH and heat treatments as a function of soaking time in the SBF, compared with that of the untreated alloy. While the untreated Ti–6Al–4V alloy revealed the Ti₂p doublet peaks of Ti₂p1/2 at 453.5 eV and Ti₂p3/2 at 459.5 eV ascribed to the Ti–Ti bond, the treated alloy (0 h) showed these peaks at slightly higher binding energies of 458.2 and 464.0 eV, which were ascribed to
the Ti–O bond [28]. This Ti–O bond is due to the amorphous sodium titanate formed on the alloy surface after NaOH and heat treatments, as implied in the previous studies [9,10,12,14]. These peaks were unchanged in energy positions after soaking in the SBF, but disappeared completely after 120 h.

Figs. 2 and 3 show the Al_{2p} and V_{2p} XPS spectra of the surface of the Ti–6Al–4V alloy subjected to the NaOH and heat treatments as a function of soaking time in the SBF, compared with that of the untreated alloy. The Al_{2p} and V_{2p} peaks at 72.35 and 511.9 eV on the untreated alloy were not detected after NaOH and heat treatments nor revealed after soaking in the SBF.

Fig. 4 shows the Na_{1s} XPS spectra of the surface of the Ti–6Al–4V alloy subjected to the NaOH and heat treatments as a function of soaking time in the SBF, compared with that of the untreated alloy. The treated alloy (0 h) showed a strong Na_{1s} peak at 1071.3 eV due to the sodium titanate on its surface. This peak appreciably decreased the intensity after soaking in the SBF for 0.5 h, and disappeared completely after 120 h.

Fig. 5 shows the O_{1s} XPS spectra of the surface of the Ti–6Al–4V alloy subjected to the NaOH and heat treatments as a function of the soaking time in the SBF, compared with those of the Ca_{3}Ti_{2}O_{7} and the HA. The

Fig. 1. Ti_{2p} XPS spectra of the surface of the Ti–6Al–4V alloy subjected to the NaOH and heat treatments as a function of soaking time in the SBF, compared with that of the untreated Ti–6Al–4V alloy.

Fig. 2. Al_{2p} XPS spectra of the surface of the Ti–6Al–4V alloy subjected to the NaOH and heat treatments as a function of soaking time in the SBF, compared with that of the untreated Ti–6Al–4V alloy.
Ca\textsubscript{2p} peaks of Ca\textsubscript{2p1/2} at 346.7 eV and Ca\textsubscript{2p3/2} at 350.1 eV were first detected after a soaking period in the SBF of 0.5 h. The intensities of these peaks were low up to a soaking period of 72 h and binding energies agreed well with those of the Ca\textsubscript{2p1/2} and Ca\textsubscript{2p3/2} respectively, which agreed well with those of the HA. After 120 h, the intensities of these peaks increased abruptly.

Fig. 7 shows the P\textsubscript{2p} XPS spectra of the surface of the Ti–6Al–4V alloy subjected to the NaOH and heat treatments as a function of the soaking time in the SBF, compared with that of the HA. A weak P\textsubscript{2p} single peak was first detected at 133.1 eV after a soaking period of 72 h. The binding energy of this peak agreed well with that of the HA. The intensity of this peak abruptly increased after 120 h.

Fig. 8 shows the TF-XRD patterns of the surface of the Ti–6Al–4V alloy subjected to the NaOH and heat treatments as a function of soaking time in the SBF, and those of the untreated alloy and the HA as references. In contrast with the untreated alloy which revealed merely the XRD peaks of Ti, the treated alloy (0 h) revealed broad XRD patterns in addition, including small peaks ascribed to crystalline sodium titanate (Na\textsubscript{2}Ti\textsubscript{3}O\textsubscript{7}) and rutile (TiO\textsubscript{2}), around 2\theta = 23 to 31° and 48°. The origin of these broad XRD patterns is an amorphous sodium titanate [9,10,12,14]. The treated alloy showed new XRD peaks ascribed to crystalline apatite after soaking in the SBF for 120 h, which increased the intensity after 168 h. The XRD pattern of apatite was fairly broad in comparison with that of HA, indicating that this apatite has bonelike defective structure with low crystallinity [31].

Fig. 9 shows the pH and element concentrations of the SBF as a function of the soaking time of the Ti–6Al–4V alloy subjected to the NaOH and heat treatments. The Na concentration and simultaneously the pH were observed to steeply increase during soaking periods up to 72 h. The Ca and P concentrations thereafter decreased gradually with increasing soaking time. Neither the Ti nor alloying species of Al and V were detected in the SBF due to the soaking of the alloy.
4. Discussion

The Ti–6Al–4V alloy was shown above to produce an Al- and V-free sodium titanate layer, which mainly exists as an amorphous phase with a small proportion of crystalline sodium titanate (Na$_2$Ti$_3$O$_7$) and rutile (TiO$_2$) on its surface by NaOH and heat treatments (Figs. 1–3, 5, and 8). On immersion in the SBF, the treated alloy was shown to form Ti-OH groups within 0.5 h (Fig. 5), and incorporate the calcium ions on its surface within 0.5 h (Fig. 6) and the phosphate ions within 72 h (Figs. 5 and 7). The alloy formed thereafter, is the crystalline apatite within 120 h (Figs. 5–8). Therefore, the Ti–6Al–4V alloy subjected to NaOH and heat treatments appears to form the apatite layer in the SBF via the following process.

When the titanium alloy was immersed in the SBF, it releases immediately a large amount of Na$^+$ ions into the SBF via exchange with H$_3$O$^+$ ions and causes an appreciable pH increase in the fluid (Figs. 4 and 9). As a result of this ion exchange, the alloy formed Ti-OH groups on its surface. The Ti-OH groups then immediately incorporates calcium ions in the fluid to form a calcium titanate. The calcium titanate incorporates the phosphate ions in the fluid to form an amorphous calcium phosphate, which later converts into crystalline apatite, growing spontaneously by consuming the calcium and phosphate ions in the fluid (Figs. 5–9).

These results indicate that the Ti-OH groups formed on
the alloy surface induce the apatite formation indirectly, by forming a calcium titanate and an amorphous calcium phosphate. This process of apatite formation on the alloy is in the same manner as on pure titanium metal [25,26]. This is because the Ti–6Al–4V alloy releases its alloying species of Al and V during the NaOH treatment, and thereby forms an Al- and V-free sodium titanate on its surface, which is qualitatively the same as that on pure titanium metal by NaOH and heat treatments [32].

The formations of calcium titanate and amorphous calcium phosphate are supposed to be the results of electrostatic interactions between the Ti-OH groups on the titanium alloy and the calcium and phosphate ions in the SBF. The pH of the SBF (7.40), which was raised by the immersion of alloy (Fig. 9), is much greater than the isoelectric point of titania (≈5.8) [33]. Around such a high pH, the Ti-OH groups have to dissociate, repolymerize and condense into negatively charged units of titania [33]. The negatively charged units of titania are postulated to interact selectively with the positively charged calcium ions in the fluid to form a calcium titanate (Fig. 6). Interestingly, the calcium titanate is present on the alloy surface for a long soaking interval (Fig. 6). During this interval, the calcium titanate may accumulate the calcium ions to gain a positive charge, and then interact with the negatively charged phosphate ions in the fluid to form an amorphous calcium phosphate (Figs. 5–7).

The amorphous calcium phosphate was shown to convert into crystalline apatite, which can be explained by the stabilities of calcium phosphates in aqueous media. HA (Ca_{10}(PO_{4})_{6}(OH)_{2}) has a lower solubility in water than any other calcium phosphate, i.e. it is the most stable form of calcium phosphate in aqueous media around pH 7.40 [34]. Namely, calcium phosphates other than HA have to eventually stabilize in the HA. Once formed, the crystalline apatite spontaneously grows by consuming the calcium and phosphate ions in the SBF, as the SBF is highly supersaturated with respect to the apatite (Figs. 5–9) [35].
Fig. 9. The pH and Na, Ca, and P concentrations of the SBF as a function of soaking time of the titanium alloy subjected to the NaOH and heat treatments.

5. Conclusions

The NaOH and heat treatments of the Ti–6Al–4V alloy produce an Al- and V-free amorphous sodium titanate surface layer on the surface. On immersion in the SBF, the alloy forms Ti–OH groups on its surface by exchanging Na⁺ ions from the surface sodium titanate layer with H₂O⁺ ions in the fluid. The Ti–OH groups on the alloy thereafter induce the apatite formation indirectly, by forming a calcium titanate and an amorphous calcium phosphate. The initial formation of the calcium titanate may be attributable to the electrostatic interaction of negatively charged units of titania dissociated from the Ti–OH groups with the positively charged calcium ions in the SBF. The calcium titanate is postulated to gain a positive charge with increasing soaking time to interact with the negatively charged phosphate ions in the SBF, consequently forming an amorphous calcium phosphate, which later stabilizes into crystalline apatite.

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