Acta Biomaterialia 7 (2011) 1379–1386

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Bone bonding bioactivity of Ti metal and Ti–Zr–Nb–Ta alloys with Ca ions incorporated on their surfaces by simple chemical and heat treatments

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ARTICLE INFO

Article history:
Received 2 July 2010
Received in revised form 17 September 2010
Accepted 20 September 2010
Available online 29 September 2010

Keywords:
Titanium alloy
Bioactive treatment
Apatite
Bone bonding
In vivo

ABSTRACT

Ti15Zr4Nb4Ta and Ti29Nb13Ta4.6Zr, which do not contain the potentially cytotoxic elements V and Al, represent a new generation of alloys with improved corrosion resistance, mechanical properties, and cytocompatibility. Recently it has become possible for the apatite forming ability of these alloys to be ascertained by treatment with alkali, $CaCl_2$, heat, and water (ACaHW). In order to confirm the actual in vivo bioactivity of commercially pure titanium (cp-Ti) and these alloys after subjecting them to ACaHW treatment at different temperatures, the bone bonding strength of implants made from these materials was evaluated. The failure load between implant and bone was measured for treated and untreated plates at 4, 8, 16, and 26 weeks after implantation in rabbit tibia. The untreated implants showed almost no bonding, whereas all treated implants showed successful bonding by 4 weeks, and the failure load subsequently increased with time. This suggests that a simple and economical ACaHW treatment could successfully be used to impart bone bonding bioactivity to Ti metal and Ti–Zr–Nb–Ta alloys in vivo. In particular, implants heat treated at 700 °C exhibited significantly greater bone bonding strength, as well as augmented in vitro apatite formation, in comparison with those treated at 600 °C. Thus, with this improved bioactive treatment process these advantageous Ti–Zr–Nb–Ta alloys can serve as useful candidates for orthopedic devices.

1. Introduction

Titanium (Ti) and its alloys are the most popular materials for orthopedic and dental implants because of their superior biocompatibility, excellent corrosion resistance, and good mechanical properties. However, they are essentially bioinert materials that, after implantation in the living body, are merely encapsulated by fibrous tissue that isolates them from the surrounding tissue. On the other hand, orthopedic load-bearing devices such as total hip prostheses require direct bonding between living bone and the implant. Hence, various methods have been developed to promote bone in-growth and implant fixation for Ti and its alloys [1,2], including physical modification of the implant design, modification of the surface topography, and chemical modification of the material composition and structure. Among these methods, plasma sprayed hydroxyapatite coating is one of the most extensively investigated methods, and its efficiency has been confirmed by many reports [3,4].

In the past decade we have developed a chemical and heat treatment method to produce bioactive Ti [5–7]. This method can be used to create a long-lasting bioactive layer on the surface of Ti and its alloys, allowing bonding with living bone via a spontaneously formed apatite layer. In this method the implants are simply immersed in aqueous solutions before heat treatment, and the bonding effects extend homogeneously throughout the irregular structure of the implant. This method is considered superior to the conventional hydroxyapatite plasma spray method, wherein the coating tends to be applied to the most superficial areas, thereby resulting in uneven and inadequate treatment. This alkali and heat treatment was applied to a porous commercially pure Ti (cp-Ti) surface layer on an artificial hip prosthesis made of a Ti6Al2Nb1Ta alloy, and its effectiveness was confirmed in clinical trials in Japan [8]. In fact, this bioactive artificial hip joint was approved for clinical use in 2007 (AHFIX, Japan Medical Materials Co., Japan).

We have also reported that our chemical and heat treatment is effective for Ti alloys (Ti6Al4V, Ti15Mo5Zr3Al, and Ti6Al2Nb1Ta) [9–11]. However, these Ti alloys contain aluminum (Al) and vanadium (V), which are suspected of being cytotoxic [12–14]. In this

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doi:10.1016/j.actbio.2010.09.026

context, the new generation of Ti alloys without V and Al [14], such as Ti15Zr4Ta4Nb and Ti29Nb13Ta4.6Zr, offers a promising alternative. Ti15Zr4Ta4Nb has been reported to show much better corrosion resistance, mechanical properties, and cytocompatibility than Ti6Al4V [15]; furthermore, Ti29Nb13Ta4.6Zr has been reported to show a lower Young's modulus and cytotoxicity than Ti6Al4V and the same cytotoxicity as cp-Ti [14,16]. Unfortunately, these new generation Ti alloys cannot be endowed with in vitro apatite forming ability by conventional chemical and heat treatment.

Instead, we recently found that Ti15Zr4Ta4Nb and Ti29Nb13Ta4.6Zr can be endowed with in vitro apatite forming ability by treatment with NaOH, $CaCl_2$, heat, and water (ACaHW). In vitro examination showed faster and greater apatite formation on the obtained calcium-modified titanate surface in simulated body fluid (SBF), with ion concentrations nearly equal to those of human blood plasma [17,18]. In this treatment calcium hydrogen titanate is formed after treatment of the Ti surface with NaOH and $CaCl_2$. Subsequent heat treatment transforms the calcium hydrogen titanate into calcium titanates and rutile [17,19]. The final water treatment causes a remarkable increase in in vitro apatite forming ability on account of the increasing mobility of the Ca^{2+} ions via incorporation of H_3O^+ ions in the calcium titanate [17]. These results lead us to expect superior in vivo bioactivity when the ACaHW treatment is applied [20]. In the present study, to confirm the in vivo bioactivity of ACaHW-treated cp-Ti, Ti15Zr4Ta4Nb, and Ti29Nb13Ta4.6Zr alloys, the biomechanical performance was investigated by histological examination and tensile strength testing using animal models [21].

2. Materials and methods

2.1. Implant preparation

Plates of size $15 \times 10 \times 2$ mm were prepared from cp-Ti (Ti > 99.5 mass%), Ti15Zr4Ta4Nb (Kobe Steel Ltd.; Ti balance, Zr 14.51, Nb 3.83, Ta 3.94, Pd 0.16, O 0.25 mass%), and Ti29Nb13Ta4.6Zr (Institute for Materials Research, Tohoku University; Ti balance, Nb 28.8, Fe 0.03,Ta 11.7, Zr 4.65, O 0.08, N 0.01, C 0.01 mass%). The plates were polished with a No. 400 diamond plate, then washed with acetone, 2-propanol, and ultrapure water in an ultrasonic cleaner for 30 min each, and finally dried at 40 °C. For bioactivation the plates were first soaked in 10 ml of 5 or 1 M aqueous NaOH solution at 60 °C for 24 h (alkali treatment). After removal from the solution they were gently rinsed with ultrapure water for 30 s and dried at 40 °C. The plates were subsequently soaked in 20 ml of 100 mM $CaCl_2$ solution at 40 °C for 24 h ($CaCl_2$ treatment), and then washed and dried in a similar manner. Next, they were heated to 600 °C (ACaH600 W) or 700 °C (ACaH700 W) at a rate of 5 °C min^{-1} in an electrical furnace in air and kept at that temperature for 1 h, followed by natural cooling. After the heat treatment they were soaked in 20 ml of ultrapure water at 80 °C for 24 h, and then washed and dried (water treatment). The concentrations of NaOH and the temperatures used for the heat treatment are listed in Table 1. In the present study we did not use ACaH600W-treated Ti29Nb13Ta4.6Zr because the Ti29Nb13Ta4.6Zr alloy had not showed apatite forming ability in SBF on ACaH600W treatment in a preliminary study. Untreated plates were used as controls in the animal experiments. Thus, a total of eight different types of plates were implanted.

Table 1
Conditions of treatment with $CaCl_2$, heat, and water after treatment with NaOH.

	Concentration of NaOH solution (60 °C 24 h)	Heat treatment temperature (1 h)
cp-Ti	5 mol l^{-1}	600 °C
	5 mol l^{-1}	700 °C
Ti15Zr4Ta4Nb	5 mol l^{-1}	600 °C
	5 mol l^{-1}	700 °C
Ti29Nb13Ta4.6Zr	1 mol l^{-1}	700 °C

2.2. Surface analyses

The surfaces of the treated plates were analyzed by field emission scanning electron microscopy (FE-SEM) (S-4300, Hitachi Co., Tokyo, Japan) equipped with an energy dispersive X-ray (EDX) analyzer (EMAX-7000, Horiba Ltd., Kyoto, Japan). The FE-SEM and EDX analyses were carried out at accelerating voltages of 15 and 5 keV, respectively.

2.3. Apatite formation in SBF

The apatite forming abilities of the treated plates were examined by soaking them in 48 ml of SBF with ion concentrations (Na^+ 142.0, K^+ 5.0, Ca^{2+} 2.5, Mg^{2+} 1.5, Cl^- 147.8, HCO_3^- 4.2, HPO_4^{2-} 1.0, and SO_4^{2-} 0.5 mM) nearly equal to those of human blood plasma at 36.5 °C [22]. After soaking for 1 or 3 days the plates were removed, gently rinsed with ultrapure water for 30 s, and dried at 40 °C. Apatite formation on their surfaces was examined by FE-SEM and EDX.

2.4. Animal study

The plates were conventionally sterilized using ethylene oxide gas and implanted into the metaphyses of the tibiae of mature male Japanese white rabbits weighing 2.8–3.5 kg. The surgical methods used have been described previously [5,10,21,23]. Briefly, the rabbits were anesthetized with an intravenous injection of sodium pentobarbital (0.5 ml kg^{-1}), an intramuscular injection of ketamine hydrochloride (10 mg kg^{-1}), and local administration of a solution of 1% lidocaine. A 3 cm long longitudinal skin incision was made on the medial side of the knee and the fascia and periosteum were incised and retracted to expose the tibial cortex. Using a dental burr, a 16×2 mm^2 hole was made from the medial to the lateral cortex running parallel to the longitudinal axis of the tibial metaphyses, as shown in Fig. 1A. After irrigating the hole with saline, the plates were implanted in the frontal direction, perforating the tibia and protruding from the medial to lateral cortex. The fascia and skin were closed in layers and the same surgical procedures were performed bilaterally.

The animals were housed individually in standard rabbit cages and fed standard rabbit food and water ad libitum. Each rabbit was killed with an overdose of intravenous sodium pentobarbital at 4, 8, 16, and 26 weeks after implantation; a total of 128 rabbits were used (eight plates of each type). The Kyoto University guidelines for animal experiments were observed in this study.

2.5. Measurement of detachment failure load

After death the segments of the proximal tibial metaphyses containing the implanted plates were harvested and prepared for the detachment tests [21]. All samples were kept moist after harvesting. The bone tissue surrounding the plates was carefully removed on both sides and at the ends using a dental burr to remove periosteal bone growth. Traction was applied vertically to the implant surface employing load test equipment (model 1310VRW, Aikoh Engineering Co. Ltd., Nagoya, Japan) at a crosshead speed of 35 mm min^{-1} (Fig. 1B–D). Specially designed hooks held the bone–plate–bone construct. The detachment failure load was measured when the plate detached from the bone. If the plate detached before the test then the failure load was defined as 0 N.