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Characterization and biostability of HA/Ti6Al4V ACL anchor prepared by simple heat-treatment

Chan-Hee Park [a,1], Min Young Jung [a,1], Leonard D. Tijing [b,*], Hae Beom Lee [c], Nam Soo Kim [c], Cheol Sang Kim [a,b,**]

[a] Department of Bionanosystem Engineering, Chonbuk National University, Jeonju, Jeonbuk 561-756, Republic of Korea
[b] Division of Mechanical Design Engineering, Chonbuk National University, Jeonju, Jeonbuk 561-756, Republic of Korea
[c] College of Veterinary Medicine, Medical School, Chonbuk National University, Jeonju, Jeonbuk 561-756, Republic of Korea

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Abstract

Here, we report a simple heat-treatment process to prepare hydroxyapatite (HA)-coated Ti6Al4V anterior cruciate ligament (ACL) anchor that has good hard tissue compatibility and biostability. Heat treatment was carried out for 1.5 h at temperature range of 700–900 °C. Morphological characterization showed rougher surface and larger pore spaces as the heat treatment temperature was increased. The Ti6Al4V heat-treated at 800 °C had the highest diffused titanium phosphide formation, thus making it high in biocompatibility. For in vivo test, the most bone integration ability was obtained for heat-treatment at 800 and 900 °C. Furthermore, the HA/Ti6Al4V ACL anchor heat-treated at 800 °C had the highest amount of new bone formation. The present results suggest that an implant with complex shape like an ACL anchor could be prepared and used with an easy and low-cost technique by simple heat treatment surface modification method after dip-coating with HA.

Keywords: B. Interfaces; B. Surfaces; E. Biomedical applications

1. Introduction

Titanium-based materials are popularly used for implants by virtue of their inertness in biological media. However, when they are exposed to severe loading or shear stress, the oxide film of titanium can deteriorate and dissolve, thereby exposing the unprotected metal to corrosion. When the stable oxide films are removed by corrosion, the body fluids could not regenerate them, which present a problem due to the dissolution of metal ions [1–3]. The amount of released metal ions is reported to increase for prosthesis with rough and porous surfaces. In long term implantation time based from clinical experiments, the accumulation of metal ions in the surrounding tissues could create adverse effects to bone tissue growth [2,4,5]. To address the problem of corrosion and metal ion dissolution, surface treatment of titanium (Ti) alloy is usually employed [1]. A lot of research have been carried out to modify the surface of Ti materials and to produce biomaterials with better compatibility with living tissues. Some of the surface treatment methods include anodic oxidation, pulse raiser deposition, chemical vapor deposition, plasma spraying, ion implantation, electrophoresis precipitation and annealing, and hot isostatic pressing (HiP) [6–9]. Among the materials used for coating, hydroxyapatite (HA) has gained attention as coating material on titanium surfaces because of its close resemblance to the natural minerals of bones and teeth [10], and it minimizes foreign body reaction to the metals, thereby allowing osteoconductive properties for direct bone bonding [11,12]. Significant research activities have been associated with the development of HA coatings.

* Corresponding author. Tel.: +82 63 270 4284; fax: +82 63 270 2460.
** Corresponding author at: Division of Mechanical Design Engineering, Chonbuk National University, Jeonju, Jeonbuk 561-756, Republic of Korea. Tel.: +82 63 270 4284; fax: +82 63 270 2460.
E-mail addresses: ltijing@jbnu.ac.kr (L.D. Tijing), chskim@jbnu.ac.kr (C.S. Kim).
[1] These authors contributed equally to this work.

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Kim and Ducheyne [13] reported that a stable and bone-bonding Ti phosphide (TiP) layer at the HA–Ti interface can be obtained by a vacuum sintering process of electrophoretically deposited HA coating on titanium alloys. One widely accepted method for depositing HA coatings on titanium-based implants is plasma spraying, but it suffers some drawbacks such as high-temperature process and difficulty in coating HA evenly. Furthermore, plasma-sprayed HA coating is reported to have problems in its long term stability due to absorption of the implant [14]. The plasma-spraying technique is also not effective for coating tiny dental implants with a complex shape [15]. To overcome these drawbacks, many HA coating processes have been investigated with the aim of improving the long term hard tissue compatibility and biostability of the HA-coated implant material, and providing a simple and economical coating process that can be used for complex-shape implants.

Here, a low-cost and simple surface modification technique by heat treatment of titanium alloy material was studied in order to achieve stable bone bonding at the interface between bone and titanium alloy implant. In the present study, a new anterior cruciate ligament (ACL) anchor made of titanium alloy (i.e., Ti6Al4V) was investigated for its hard tissue compatibility and biostability. An ACL anchor is a device that is designed for artificial ligament fixation between the femur and tibia as a substitute for an injured and ruptured ligament. The present ACL anchor made of Ti6Al4V was dip-coated with HA paste and was subjected to heat treatment. Ti6Al4V has higher mechanical strength than pure titanium (Ti) but is also known to cause an increase in stress shielding because of its higher modulus of elasticity [16]. In this paper, a dip-coating technique was used to coat the Ti6Al4V ACL anchor with HA paste (referred herein as HA/Ti6Al4V) instead of using HA powder, and was subjected to heat-treatment at different temperatures (i.e., 700–900 °C). The objectives of the present study were to investigate the physico-chemical surface properties of a heat-treated HA/Ti6Al4V ACL anchor, and to determine its hard tissue compatibility and biostability.

2. Materials and methods

2.1. Specimen preparation

Cylindrical specimens (Grade 2, ASTM F67, length = 5 mm and diameter = 6 mm) were used for the investigation of the surface properties of commercial grade Ti6Al4V. The cylindrical specimens were mechanically polished in sequence by SiC grit papers (# 1200, # 2000) and then with alumina powder (0.3 μm, 0.05 μm), and then ultrasonically cleaned three times in biotergent and distilled water in accordance with the standard procedures for metallic implant devices [17,18]. The specimens were coated with HA ($Ca_5(OH)(PO_4)_3$, MW = 502.31 g mol^{-1}, Fluka Biochemika Sigma Aldrich, No. 55497) to an approximately 200 μm in thickness by dipping them in an HA paste (mixture of 0.06 g HA and 1 ml distilled water) for 3–5 min and subsequently dried in ambient condition. Heat-treatment of the uncoated and HA-coated specimens was carried out for 1.5 h under protective Argon atmosphere in vacuum (10^{-2} Torr) at different temperatures: 700, 800, 850, and 900 °C. The adhered HA layers on the metal surfaces were then exfoliated with strong water jet spray, and the specimens were dried on a clean bench.

2.2. Characterization and measurement

The surface morphology and structure of the specimens were characterized by scanning electron microscopy (SEM, Hitachi X-650, Japan), atomic force microscopy (AFM, Autoprobe LS, PSI, USA), X-ray diffraction (XRD, Rigaku D/MAX-IIIA, Japan), and scanning Auger electron spectroscopy (SAES, VG Escalab 210, UK). Typical SAES instrument conditions for the interface analysis were: vacuum $< 6 \times 10^{-11}$ Torr, 6 keV electron beam energy, and 0.3 μA/(500 × 500 μm^2) electron beam current density. The tensile strength and hardness of the heat-treated Ti6Al4V specimens ($n = 5$) were measured by Instron mechanical tester and Vicker's hardness tester, respectively.

2.3. In vivo test

In order to evaluate the in vivo behavior of the heat-treated HA/Ti6Al4V samples, the same dip-coating preparation and heat-treatment procedure as stated above were carried out using an ACL anchor specimen (Fig. 1a). The design parameters and initial tests of the present ACL anchor are reported in our previous study [19]. The in vivo test was divided into the bone integration and biological stability tests. For bone integration test, nine adult New Zealand rabbits all aged 9 months old were used for experiments. They were anesthesized with intramuscular injections of Ketamine (10 mg/kg of animal weight) and Rompun (0.15 mg/kg of animal weight). Additionally, 1 ml of 2% Lidocaine (1:100,000 epinephrine) was administered to the cortical bone where the ACL anchor was to be inserted. Four implant specimens were implanted into the cortical bone of each rabbit. The operation was conducted according to the surgical protocol of Branemark's implant system. The round drill, 2.0-mm twist drill, 2.7-mm pilot drill, and 3.0-mm twist drill were used consecutively, including careful drilling with a low rotary drill (never exceeding 2000 rpm) and profused saline cooling. After four weeks of insertion, the rabbits were sacrificed with a fatal amount of pentobarbital injection. Immediately after sacrifice, each specimen was subjected to a removal torque test using a torque gauge instrument (Shinsung Co., Korea).

Moreover, biological stability test was conducted in order to evaluate the cytotoxicity and inflammatory reaction of the heat-treated ACL anchors by implanting them into the cortical bone of the femur of a dog under local anesthesia. Blood tests, which include complete blood count (CBC) and chemistry screening (CS), were conducted for every two weeks and after six weeks, the dog was euthanized by intravenous administration of

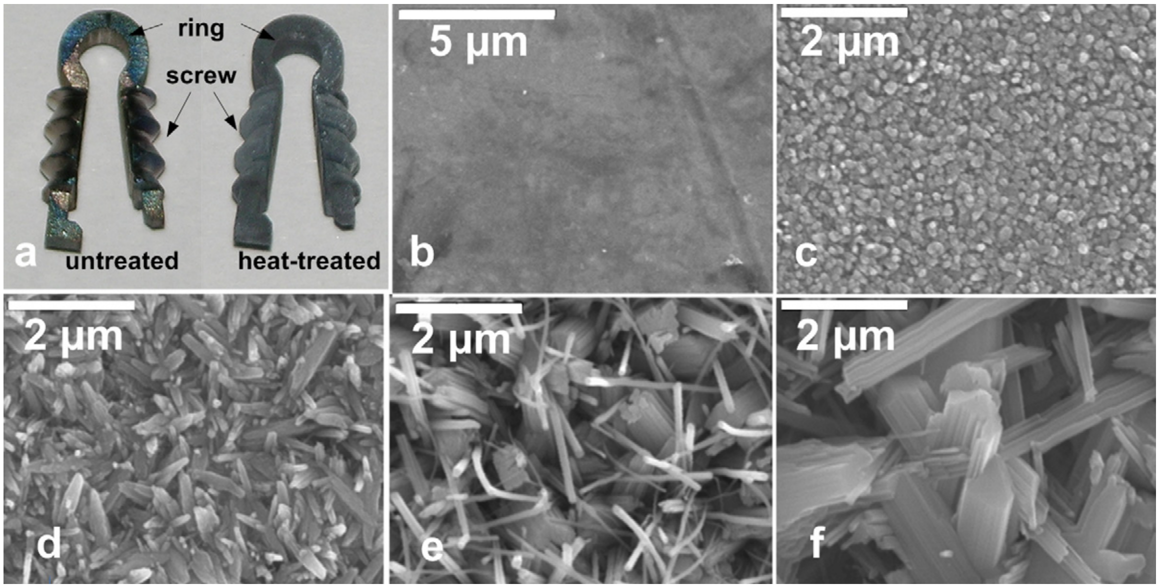

Fig. 1. (a) Photo of untreated and heat-treated HA/Ti6Al4V ACL anchor. SEM images of (b) untreated, and heat-treated HA/Ti6Al4V samples at: (c) 700 °C, (d) 800 °C, (e) 850 °C, and (f) 900 °C.

sodium pentobarbital (Entobar Inj®, Han Lim Pharm, Korea). Histological observation was done on the hard tissue specimen and on the removed calcified tissue to determine any formation of osteoblast and new bone. Each sample was fixed with 10% formalin, demineralized with nitric acid for 3–5 days, and processed routinely for light microscopy. The 5-μm thick sections were stained with Masson trichrome and observed under light microscope. The Chonbuk National University guidelines for animal experiments were observed in this study.

3. Results and discussion

3.1. Surface characterization and mechanical test

Fig. 1b–f shows the surface morphologies of the untreated and heat-treated (700–900 °C) HA/Ti6Al4V specimens. The heat-treated specimens had completely different surface topographies than the untreated specimen, which had a smooth surface. The heating (Fig. 1c–f) led to the formation of porous and rough surfaces. Needle-like structures started to emerge at 800 °C on the surface, and at 900 °C, some rectangular crystal segments with bigger width were formed. As the heat-treatment temperature was increased, the pore spaces also increased with grain growth. The heat-treatment at 800 °C (Fig. 1d) of HA/Ti6Al4V specimen obtained a mean surface roughness of about 11 nm and a pore lateral size of 40 nm (see Table 1). Numerous reports have shown that both the early fixation and long-term mechanical stability of the implant can be improved by a high roughness profile compared to smooth surfaces [20,21]. The high degree of surface roughness (i.e., increased surface area) provide more area for the bonding to occur, which could lead to stronger implant–bone bonding [22,23]. Here, the morphological change of the surface was mostly due to Ti oxides formed with residual oxygen in the chamber as shown in the SAES depth profile (Fig. 2c). Table 1 shows increased oxide layer at the surface of the newly formed compound as the heat-treatment temperature was increased. The higher oxide content made the Ti6Al4V material harder yet more brittle and fragile (see Fig. 3). The phosphorus (P) content in TiP layers showed a decreasing trend as the heat-treatment temperature was increased. The presence of phosphorus layer on the surface enhances the bone bonding strength. The maximum concentration of diffused phosphide layer was achieved at heat-treatment of 800 °C. It is generally known

Table 1
Surface properties of untreated and heat-treated HA/Ti6Al4V at different temperatures.

Properties	c.pTi6Al4V	700 °C	800 °C	850 °C	900 °C
Oxide layer thickness (nm)	~4.0	~200	~600	~1000	>2000
Mean surface roughness (nm)	~4	~7	~11	~123	~166
Mean pore size (lateral/vertical, nm/nm)	–	~10/100	~40/300	~70/400	~100/700
Maximum atomic concentration of P in TiP layers (%)	–	–	~7.5	~2.0	<1.0

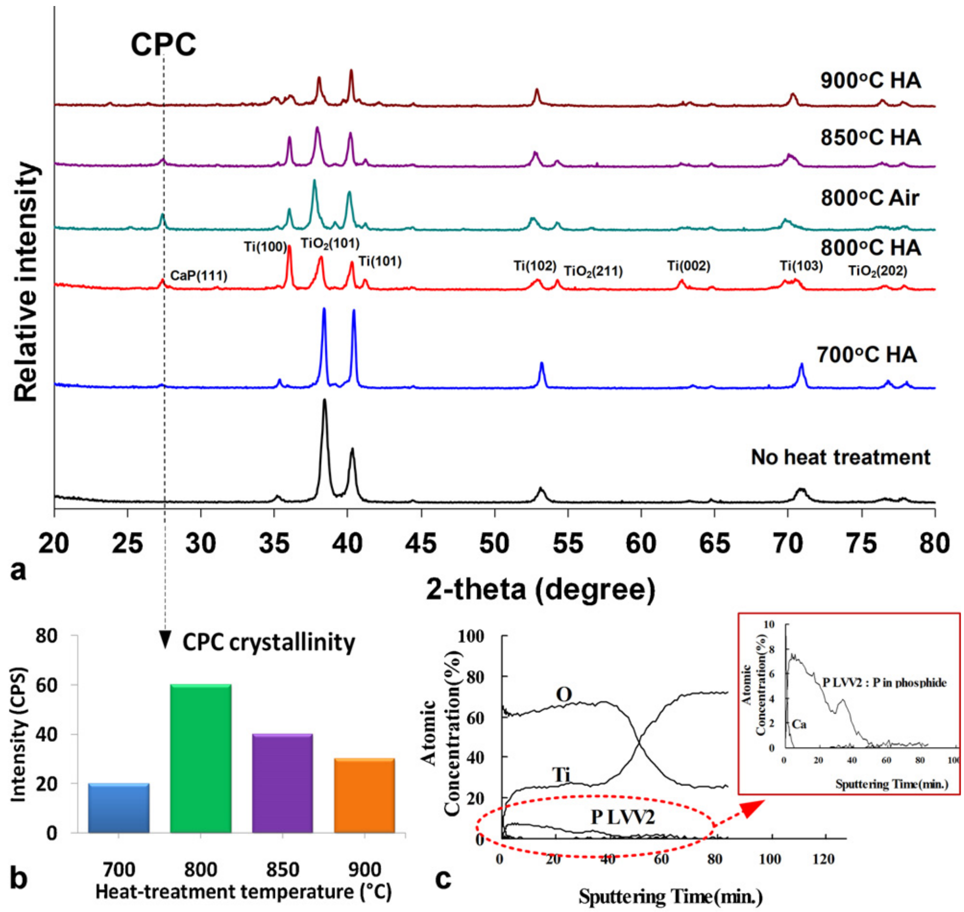

Fig. 2. (a) XRD spectra, (b) CPC crystallinity, and (c) SAES depth profile (inset: enlarged image of dotted area) of untreated and heat-treated HA/Ti6Al4V at different temperatures.

that the surface topography of an implant is important for biological activity. Based on the results, it suggests that the present simple heat treatment technique is useful for controlling the topography and the chemical composition of the surface, which has a strong influence on the adhesion, morphology, and orientation of cells and the absorption of biomolecules [24].

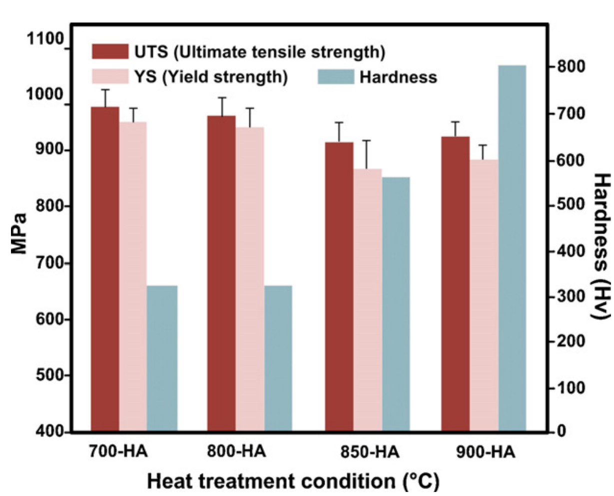

Fig. 3. Tensile strength and hardness of HA/Ti6Al64V heat-treated at different temperatures.

Heat treatment affects the crystallinity of the HA coating, which could consequently affect the bone healing and formation of new bone [25]. HA is known to improve the osseointegration following implantation [26]. Fig. 2a shows the XRD spectra of the untreated and heat-treated specimens at different conditions. TiO_2 peaks can be observed for all cases. The vertical dotted line designates the position of the calcium phosphate compounds (CPC, (1 1 1)) which was observed for all heat-treated cases but at different diffused concentrations (Fig. 2b). The presence of CPC was also confirmed by SAES (Fig. 2c). The amount of CPC crystals (Fig. 2b) on the surface determines the material's biocompatibility and resorption rate [27]. HA coatings with high degrees of crystallinity show low-dissolution rates in vitro and less resorption and more direct contact in vivo [28,29]. The highest value of CPC crystallinity was obtained at 800 °C, which suggests good biocompatibility.

Fig. 3 shows the results of tensile and hardness tests of HA/Ti6Al64V specimens heat-treated at different temperatures. HA/Ti6Al64V showed decreasing tensile strength as the heat treatment temperature was increased. Heat-treatment at 700 °C and 800 °C showed the highest tensile strengths among the specimens. At further elevation of temperature to 850 and 900 °C, the tensile strength decreased. This is mainly because of the wider pore spaces and rougher surface when the temperature was increased. On the other hand, the hardness of Ti6Al64V generally increased as the heat-treatment temperature was increased. The significant increase in hardness of heat treated specimens at 850 and 900 °C is believed to be primarily because of their thicker oxide layers, which cause brittleness and fragility [30]. This fragility due to high thermal oxidation may give rise to serious weaknesses in implants or to stress shielding by Ti implants inducing bone resorption. From the mechanical test result, it is obvious that at a heat-treatment of 800 °C, one can achieve a high tensile strength, and a less brittle material.

3.2. In vivo test

Fig. 4a shows the bone integration test results showing the removal torque with respect to the heat-treatment condition. HA/Ti6Al4V ACL anchor heat-treated at 800 and 900 °C showed the highest removal torque values, i.e., high bonding strength. At 800 °C, the reason could be because of its high amount of diffused element P, which could induce the formation of new bone. At 900 °C, the chemical composition and surface morphology such as increased roughness, which are suitable for growing new bone, could be the reasons for its high resistance to removal. A rough implant surface has previously been shown to be of importance for bone-to-implant retention. This has been demonstrated in animal experiments by pull-out, push-out and torque studies [31–33]. It is known that a fibrous scar tissue band occurs at the graft–bone interface after implantation in the bone tunnel that influences graft stability in the host bone tunnel. Different techniques have been developed to promote and enhance graft osseointegration within the host bone tunnel [34]. Lee et al. [35] reported that composite HA/BG coating facilitated adhesive attachment of surrounding soft tissue to a Ti6Al4V implant surface. In this study, we coated a complex-shape Ti6Al4V ACL anchor with HA paste and subjected it to a simple heat-treatment process. After 4 weeks, the HA/Ti6Al4V ACL anchor heat-treated at 800 °C showed the highest amount of new bone formation and bone contact rate (Fig. 4b) among the tested samples. When ACL anchor was heat-treated above 800 °C, the amount of new bone and bone contact rate were relatively low. The highest bone formation and bone contact rate were obtained at a heat-treatment temperature of 800 °C, suggesting that its biocompatibility was very good at this treatment condition. On the other hand, the implant heat treated without HA paste at 800 °C (control) showed little amount of bone growth and bone contact rate (Fig. 5a). Based on the results, heat treatment of Ti6Al4V with HA coating could definitely help in the formation of new bone. HA/Ti6Al4V heat-treated at 800 °C (Fig. 5b) shows fibrous tissue growth on the implant. Fibrous connective tissue filled the upper part of the inside of the implant. At the ring of the ACL anchor, a lot of bone marrow was discovered, with relatively good bone union. Fig. 5c (heat-treated at 850 °C) shows collagen fibers filling up the upper part of the implant. There was no inflammatory reaction observed. At 900 °C (Fig. 5d), fibrous tela connectiva also filled the space, but the formation of bone tissue was weak. The HA/Ti6Al4V ACL anchors heat-treated at different temperatures all revealed no inflammatory reaction and abnormal response after six weeks, which is also supported by the blood test results (not shown here), showing values within range of the standard level of blood composition, suggesting no hematological or biochemical abnormalities. Here, the histological findings revealed that the heat-treated HA/Ti6Al4V ACL anchor stimulated the formation of new bone and has good osseointegration within the host bone [34].

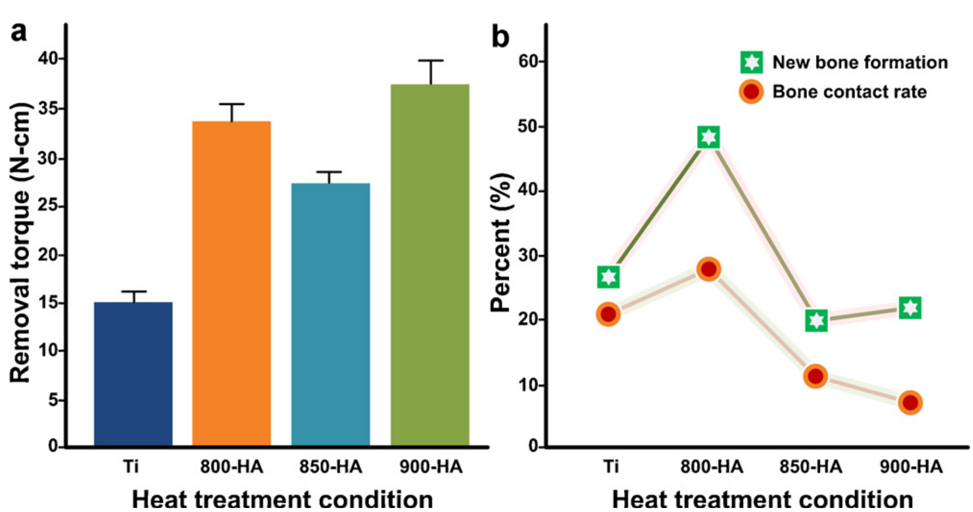

Fig. 4. (a) Removal torque and (b) new bone formation and bone contact rate after in vivo test of untreated and heat-treated HA/Ti6Al64V ACL anchor.

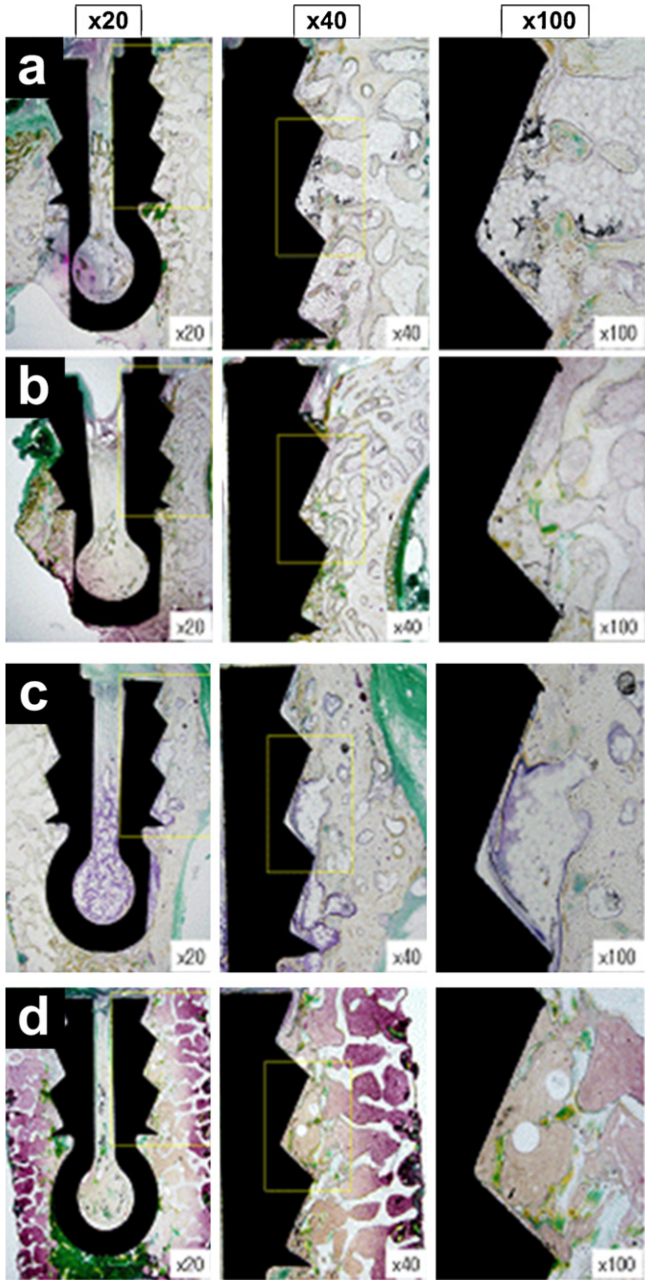

Fig. 5. Histological characterization of untreated and heat-treated HA/Ti6Al4V anchor at different temperatures: (a) Ti6Al4V implant without HA coating and heat-treated at 800 °C; HA/Ti6Al4V implant heat-treated at (b) 800 °C, (c) 850 °C, and (d) 900 °C.

4. Conclusions

The following are the conclusions drawn from this study:

(a) Morphological characterization of the untreated and heat-treated HA/Ti6Al4V samples showed rougher surface and larger pore spaces as the heat treatment temperature was elevated. The HA/Ti6Al4V heat-treated at 800 °C had the highest diffused titanium phosphide formation.
(b) The tensile strength of HA/Ti6Al4V decreased as the heat treatment temperature was increased with a corresponding increase in hardness.
(c) The highest CPC content was found at the heat treatment temperature of 800 °C after dip-coating with HA.
(d) The results for in vivo test showed most integration ability at heat-treatment of 800 and 900 °C. There was no specific inflammatory reaction and abnormal response for all tested samples after implantation.
(e) The HA/Ti6Al4V ACL anchor heat-treated at 800 °C had the highest amount of new bone formation.
(f) The present simple heat-treatment method may provide suitable surface modification technique to improve the biological performance of titanium implants especially those with complicated shapes and structures.

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