Influence of Applied Voltages on Mechanical Properties and In-Vitro Performances of Electroplated Hydroxyapatite Coatings on Pure Titanium

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Abstract

Titanium and its alloys have been extensively used in biomaterials due to their bone-compatible modulus, superior bio-compatibility and enhanced corrosion resistance. But the metal implants that contact the body will release ions and scraps. In this study, Hydroxyapatite (HA, Ca10(PO4)6(OH)2) was coated on a pure Ti substrate by electrochemical deposition process, which can provide a well-controlled process and product. Across various applied voltages, the electroplated HA/Ti composites were evaluated on their chemical and mechanical performances and the behaviors of in-vitro simulating tests. Highly pure HA coatings were obtained with additional voltages from 16 V to 19 V. In addition, their mechanical properties were significantly enhanced with increasing voltages. With the results of in-vitro evaluations, the electroplated HA/Ti composites is capable of serving as a biomaterial for bone substitution or implantation.

Biomaterials are synthetic materials that function in vivo or in vitro while physically in contact with the human body. Due to the coexistence with blood and body fluids, an ideal biomaterial must be biocompatible, nontoxic, stable, easily-manufactured and with proper mechanical properties.1,2 For an implantation, the main purpose is repairing the loss of tissues or organs to restore their original functions; thus, bio-compatibility is the most concerning factor.

Human tissues are roughly divided into two categories: soft and rigid. A bone is a rigid organ that constitutes parts of the skeleton. Bones further support and protect the organs of the body, enable human mobility, produce red and white blood cells and also store minerals. While bone tissues age and are damaged by external forces, they have an inherent ability to regenerate. However, an excessive bone loss requires the use of bone grafts. And while graft materials regularly originate from the same individual (autograft), a donor (allograft) or animal bone (heterograft), artificial biomaterials are attracting more and more interest in the field of tissue engineering.3,4

The metallic materials known and widely used for medical implants in bone reconstructive surgery and prosthetic treatment are stainless steel (316L), Co-Cr alloys and unalloyed Ti.5–10 However, some chronic or nickel ions could be released into the body due to metallic fatigue and corrosion, resulting in inflammation or toxicity.11 Moreover, implanted hard tissues might cause a stress-shielding effect and bone resorption reactions because of the elastic incoordination with surrounding bones. For actual biomedical applications, considering mechanical properties and stability, titanium and its alloys are still selected for replacing supports in the human body, including artificial joints, bones, stents, tooth roots and other implanted artificial substitute materials.12

Hydroxyapatite (HA, Ca10(PO4)6(OH)2) is a major composition of human bones and also a popular ceramic material used in orthopedics. After implantation, the calcium and phosphorus ions could be released and absorbed to benefit the growth of new tissues.13 HA is also a good biological carrier for the attachment of bone cells; thus, it is a functional filling material in repairing bone defects.14,15 Because of the similarity of compositions and the effects for cell growth, HA is commonly utilized to modify the surface status of implanting materials. However, its poor tenacity and adhesion limit the practical applications as well.

Studies on improving the adhesion of HA on titanium substrates by various deposition processes are widely reported.16–23 Zheng et al. reported the formation of HA/Ti composite coating on Ti-6Al-4V alloy by using a thermal spray process, resulting in the bonding strength raising from 12.9 MPa to 17.3 MPa while the titanium content increased from 0 to 60%.16 The rapid-cooling rate of thermal-sprayed HA could cause residual stress at the interface, so a coating peeling issue might occur after implanted for a period of time. Thermal gradients also could cause a composition difference, which influences the coating properties significantly.17 Adding ceramics could enhance HA coating's adhesion. For example, nearly inert ceramics, such as alumina, zirconia and titania were reported for their effects on raising the tenacity and adhesion strength of HA coating.18,19 Thus, Ti and its alloys with HA coatings could bear high loadings, increase osteogenesis efficiency, improve bonding strength, and reduce the release of metallic ions to assist the formation of newborn bones.20–22

Recently, electroplating calcium phosphate coatings attracted much interest due to their noticeable advantages, including low-temperature and well-controllable deposition, high coating stability, good coverage on complex or porous surface and low processing cost.24–26 In this study, a pure Ti substrate was modified carefully with HA coating by using an electrodeposition process. In order to evaluate their effects on bone tissues, the HA/Ti composite’s morphologies, physical and mechanical properties were investigated. Moreover, we further simulated situations in the human body by holding the HA/Ti composites in artificial body fluid.

Experimental

In-vivo and in-vitro tests are two common methods for evaluating bone substitution materials. In order to investigate the physical and chemical properties of implanted biomaterials, most studies would simulate an in-vitro test by immersing samples in an artificial body fluid with a similar composition of plasma. Improvements and animal experiments would then be proposed based on the results of simulations. After confirming that there were no adverse reactions, the materials would then be moved toward the goal of human clinical trials. Here, the in-vitro tests would be introduced to evaluate the near-practical improved efficiency of HA treatments. The experimental procedures in this study are listed as following.

Chemicals and materials.—All chemicals are of analytical grade and used as received without any further purification. Calcium nitrate tetrahydrate (Ca(NO3)2· 4H2O) and ammonium dihydrogen phosphate (NH4H2PO4) were purchased from Showa Kako Corp. Sodium...
chloride (NaCl), sodium hydrogen carbonate (NaHCO₃), sodium phosphate (Na₃HPO₄), calcium chloride (CaCl₂), magnesium chloride (MgCl₂·6H₂O), magnesium sulfate (MgSO₄·7H₂O), sodium phosphate (Na₃HPO₄·2H₂O), potassium chloride (KCl), and dextrose anhydrous (C₆H₁₂O₆) were purchased from Shimakyu’s Pure Chemicals Ltd.

Pure titanium (99.7%) foil was cut into 2 cm × 2 cm substrates. After being roughly polished with sandpaper, the Ti plates were washed thoroughly with ethanol several times and dried in an oven at 60 °C ambient.

Preparation of HA/Ti composites.—First, 4.96 g of calcium nitrate tetrahydrate was gradually added into 500 mL distilled water, and 1.438 g of ammonium dihydrogen phosphate was dissolved in the same volume of distilled water. After mixing these two solutions, 1 L electrolyte for electroplating was prepared. Then, the mixed solution was transferred to a reaction vessel, where a pure Ti plate was connected to the cathode and the anode was a platinum foil with a constant distance of 3 cm. The electrodeposition proceeded with the applied voltages designed as 7, 10, 13, 16, 19, 22, 25 V at 60 °C for 15 min and herein denoted as HA7, HA10, HA13, HA16, HA19, HA22 and HA25, respectively. Finally, the obtained HA/Ti composite plates were washed several times with distilled water to remove any possible residue and then heat treated at 600 °C in an oven for 2 h. The heating rate was set as 5 °C/min to avoid any possible cracking.

Characterization of HA/Ti composites.—The finished HA/Ti composite plates were directly characterized by an X-ray powder diffractometer (D/MAX-B, Rigaku) using a graphite monochromator with Cu ka X-ray radiation operated at 40 kV and 30 mA under 0-2θ configuration. The images of surface morphologies were observed via field emission scanning electron microscopy (FE-SEM, Hitachi S-4700N). A Fourier transform infrared spectrometer (FT-IR, Horiba FT-760) was used to identify the molecular bonding of coatings. Analytes must be removed, ground into powder, and mixed with potassium bromide (KBr) at a 1:100 ratio. The flattened samples were analyzed under a beam power ranging from 500 to 4000 cm⁻¹.

Mechanical properties influence the performances of in vivo bone substrate significantly. Excessive strength could cause native bones to abrade; in contrast, a strength inadequacy results in fragile structures. For this reason, HA/Ti composites were further examined for their abilities to resist external forces. In addition to roughness and hardness, the bonding strengths were also tested on a universal material testing machine (Shimadzu 1000 kN, Japan) in accordance with ASTM C633 and F1501-95 criteria. A gauge diameter of 11 mm was used for testing samples.

In-vitro evaluation in artificial body fluid.—To further simulate and investigate how the HA/Ti materials react and dissolve in vitro, samples were immersed in a simulated body fluid (SBF) Hank’s balanced salt solution. Table 1 lists the formula for Hank’s solution. The immersing times for HA/Ti composites in SBF were 1, 4, 7, 14 and 28 days. Recording the weight differences afterward would help us understand the degree of absorption and desorption in SBF. Thus, we can evaluate the equivalent situations that HA/Ti composites would work in vivo.

### Results and Discussion

#### Characterization of HA/Ti composites.

The XRD patterns of the obtained HA/Ti composites under different applied voltages are shown in Figure 1. As-prepared HA/Ti composites show the combined diffraction peaks of hydroxyapatite and titanium. It has been reported that the hydroxyl ions in electrolyte were beneficial for the formation of perovskite (CaTiO₃) interphase, which was the main factor for inducing HA to nucleate. Since no perovskite signal was detected, it means they were transferred to hexagonal HA structure entirely.

The FT-IR spectrometer was utilized to verify the bonding types, as shown in Fig. 2. HA7 and HA25 revealed the standard characteristic peaks of HA. There are four main function groups, including PO₄³⁻, CO₃²⁻, H₂O and OH⁻. PO₄³⁻ modes are located at 450–1200 cm⁻¹, resulting from the asymmetric P-O bonding. Between 1350 and 1580 cm⁻¹, the peaks indicate the formation of carbonated HA (Ca₁₀(PO₄)₆(CO₃)₂·CH₂O) and calcium carbonate due to the participation of exterior carbon dioxide. A wide peak at 3200–3420 cm⁻¹ is the HA function group caused by water molecules absorbed on the HA
Combined with XRD results, it was confirmed perovskite completely transferred to crystalline HA under the applied voltages.

Figure 3 shows the SEM images of electroplated HA/Ti samples. Their morphologies showed the lamellar, porous and rod-like structures, which are beneficial for cell adsorption and growth. On HA7, the HA crystals were thin and almost needle-like in structure, as shown in Fig. 3a. As the voltage increased slightly, HA crystals grew into a round shape at 10–13 V. From 16 to 25 V, Figures 3c–3f indicate a layered rod-like structure, with density and crystalline dimensions increasing with the applied voltages.

By further analyzing the chemical composition of various HA/Ti composites by EDS, four main elements were detected, including calcium, phosphorus, oxygen and titanium. Ca, P and O are the compositions of HA, and Ti is from the substrates. Combining with XRD and FT-IR results, HA coatings were confirmed deposited onto pure Ti substrates. In order to further ensure the purity of obtained HA coatings, their Ca/P ratios were calculated in Table II and compared with the theoretic value of HA, 1.67. The Ca/P ratio was 1.64 at 7 V, and increased with the applied voltages. The optimized Ca/P value was 1.666 and appeared at 19 V, while the ratio decreased again with the applied voltages exceeding 19 V.

**Mechanical properties of HA/Ti composites.**—The average roughness of HA coatings varied inversely with the increase of applied voltages, as presented in Figure 4a. The highest value was 6.33 μm at 7 V and then reduced with the voltages raising. Over 16 V, the roughness kept at the relatively low ones, around 2 μm. This is because the coatings were loose and porous at low voltages, resulting in a non-uniform thickness and high roughness. A higher applied voltage for electroplating is advantageous to dense HA coatings. Although a biomaterial with rough surface is beneficial for cell’s adsorption and growth, a loose and over-rough structure still should be avoided.

Figure 4b shows the bonding strengths of HA coatings on Ti substrates based on ASTM C-633 and F1051-95 criterion. The plot presented an increasing trend from 0.7 MPa with the applied voltages, and reached a maximum value, 2.25 MPa, at 19 V. The main reason is the high density resulting from a high voltage; so that, the coatings would grip tighter onto substrates as well. Furthermore, the results of hardness tests were drawn in Figure 4c to verify the density effect. Comparing with Figure 4a, the coating’s hardness was inversely

![Figure 3. SEM images of (a) HA7, (b) HA10, (c) HA16, (d) HA19, (e) HA22 and (f) HA25.](image)

![Figure 4. (a) Surface roughness, (b) bonding strengths, and (c) hardness of various HA coatings on pure Ti substrates.](image)
HA16 and HA25 gained the highest-efficient performance as well. moreover, the total difference was increasing with time. By 28 days, weights of each sample rose significantly after immersed for 28 days; composites immersed in BSF for 1 to 28 days. Except for HA7, the metals or polymers. Figure 5 reports the weight differences of HA/Ti composites immersed in BSF for 1, 4, 7, 14 and 28 days, respectively. Weight differences of various HA/Ti composites after immersed in BSF for 1, 4, 7, 14 and 28 days, respectively. Despite for HA7, the weights of each sample rose significantly after immersed for 28 days; moreover, the total difference was increasing with time. By 28 days, HA16 and HA25 gained the highest-efficiency performance as well.

**Summary**

The improvement of the mechanical properties of hydroxyapatite (HA) electrodeposited on pure titanium substrates and their in-vitro performances were studied in this article. According to the results of chemical analyses, the HA/Ti composites were obtained correctly with various applied voltages ranging from 7 to 25 V. Their morphologies were of porosity and rod-like structure; in addition, HA16 and HA19 got a fairly dense structure and the optimized Ca/P ratio. The investigations of mechanical properties further indicated that the HA’s densities, hardness and bonding strengths raised with the increase of applied voltages. Over 16 V, the HA/Ti composites could provide a suitable physical condition for biomaterials. After simulated immersing tests, the weighting evaluations indicated the HA/Ti coatings benefited cell’s attachment except for HA7. In summary, HA16 is a relatively suitable biomaterial for bone implantation based on our experiments.

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