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Electrolytic deposition of octacalcium phosphate/collagen composite coating on titanium alloy

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Abstract Osteointegration of titanium or its alloy with bone can be greatly improved by calcium phosphate coatings, and further enhanced by an extracellular matrix protein layer such as collagen. In this study, an octacalcium phosphate (OCP)/collagen composite coating layer on Ti6Al4V substrate was prepared using electrolytic deposition method. A layer of OCP mineral consisting of flake-like crystals was first formed on the Ti6Al4V substrate. Subsequently, mineralized collagen fibrils were deposited on the former OCP layer. These collagen fibrils were interconnected and well adhered on the OCP layer so that they were immobilized. The microstructure of the composite coating varied with collagen concentration in the electrolyte. This study could offer a possibility of fabricating a desired surface matrix on orthopedic implants to enhance bone formation and fixation of implants.

1 Introduction

Titanium and its alloys are often used as orthopedic and dental implants because of good biocompatibility and mechanical property. To improve the bioactivity of implant surface, hydroxyapatite (HA) coating is usually required because it can create a chemical integration of the implant with host bone. However, the early cell response and healing of Ca-P coating is still unsatisfactory.

The early bone regeneration depends on osteoblastic cell responses, such as adhesion, proliferation, and differentiation. Studies in developmental and cell biology have established the fact that responses of the cells are influenced to a large extent by morphology and composition of the extracellular matrix (ECM) [1, 2]. As the main component of the ECM of bone, collagen I could provide a desirable matrix for osteoblastic cell adhesion and growth [3–5].

Ca-P/collagen composite materials similar with natural bone tissue have attracted much attention in tissue engineering [6]. Farina reported that HA/collagen composite could act as ideal scaffold for osteoconduction and osteoinduction [7]. However, the challenge now is to fabricate such a composite coating on metal implant to increase the surface bioactivity. Electrochemical process is a viable method to synthesize Ca-P/collagen coating. Roveri has synthesized HA/collagen coating on titanium [8]. This composite coating seems not homogeneous on the substrate and the deposited HA particles heavily cover collagen fibrils. In addition, HA phase is considered to be almost insoluble and may mask the biological function of collagen fibrils after implantation.

Here, an electrolytic deposition method is attempted to prepare a homogeneous OCP/collagen composite coating on Ti6Al4V substrate. A pH gradient above the substrate induced by the electrode reaction is helpful to the formation of collagen fibrils integrated with OCP mineral as a coating on the metallic implant. The morphology, phase, and microstructure of the composite coating were investigated and briefly discussed.

2 Materials and methods

The working electrode was Ti6Al4V plate ($10 \times 20 \times 1$ mm^3), and the counter electrode was a platinum plate

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($15 \times 25 \times 0.1$ mm^3). The surface of the Ti6Al4V plate was polished with No. 1000 SiC paper, cleaned ultrasonically in acetone and ethanol, and finally rinsed in de-ionized water. The electrolyte used in this study was prepared by dissolving 4.5 mM $Ca(NO_3)_2$ (Shanghai Chem. Co., China) and 9 mM $NH_4H_2PO_4$ (Shanghai Chem. Co., China) in distilled water. Type I collagen (Sigma-Aldrich. Co.) was dissolved in 10 mM hydrochloric acid solution after several days stirring. And then the collagen solution was added into electrolyte at the concentration of 0.1–0.5 mg/ml and then followed by pH adjustment at 3.8 by adding ammonia solution and monitored with a calibrated pH meter (Mettler Toledo 320-S). The deposition process with a potentiostat was conducted in electrolytes with different collagen concentrations at 37°C. The distance between the counter and cathodic electrodes was fixed at 15 mm, and the deposition time was 30 min. The obtained coating was washed with de-ionized water to clear the absorptions and dried in air.

The coatings were observed using scanning electron microscope (SEM, JSM-5610LV) and transmission electron microscope (TEM, JEOL200CX). The chemical composition of the coating was detected using energy dispersed spectroscope (INCA Energy 300). The crystalline phase of the composite coating was measured using X-ray diffraction (XRD, X' Pert PRO, CuKα, 2°/min, 0.02 per step). The adhesion of the coating was evaluated by a simple tape test [9].

3 Results and discussion

Figure 1 shows the surface morphologies of the coatings deposited from electrolytes with different collagen concentrations. The presence of collagen in the electrolyte obviously influences the Ca-P crystallite morphology. The crystallites in the Ca-P layer formed from the electrolyte without collagen have a flat shape and form a homogenous Ca-P coating on the substrate (Fig. 1a). When the electrolyte contains 0.1 mg/ml collagen, the crystallites in the resulting Ca-P layer become curved and small, and no obvious collagen fibrils are observed (Fig. 1b). As the collagen concentration reaches 0.3 mg/ml in the electrolyte, collagen fibrils appear on the Ca-P layer and the surface of the collagen fibrils are covered by flake-like Ca-P minerals as mineralized ones (Fig. 1c). These mineralized collagen fibrils are immobilized on the substrate through Ca-P crystals. When the collagen concentration in the electrolyte further increases to 0.5 mg/ml, more mineralized collagen fibrils spread over the surface, and some naked collagen fibrils appear (Fig. 1d). In the present bilayer structure coating, the bottom OCP layer can spontaneously transform to HA in situ and acts as a commonly used coating which can integrate with bone tissue to promise the stability of implant. The top composite layer provides an extracellular matrix for the early stage cell adhesion and growth.

The cross-sectional SEM photograph of the coating deposited from the electrolyte with 0.3 mg/ml collagen shown in Fig. 2 reveals that the bottom Ca-P layer is

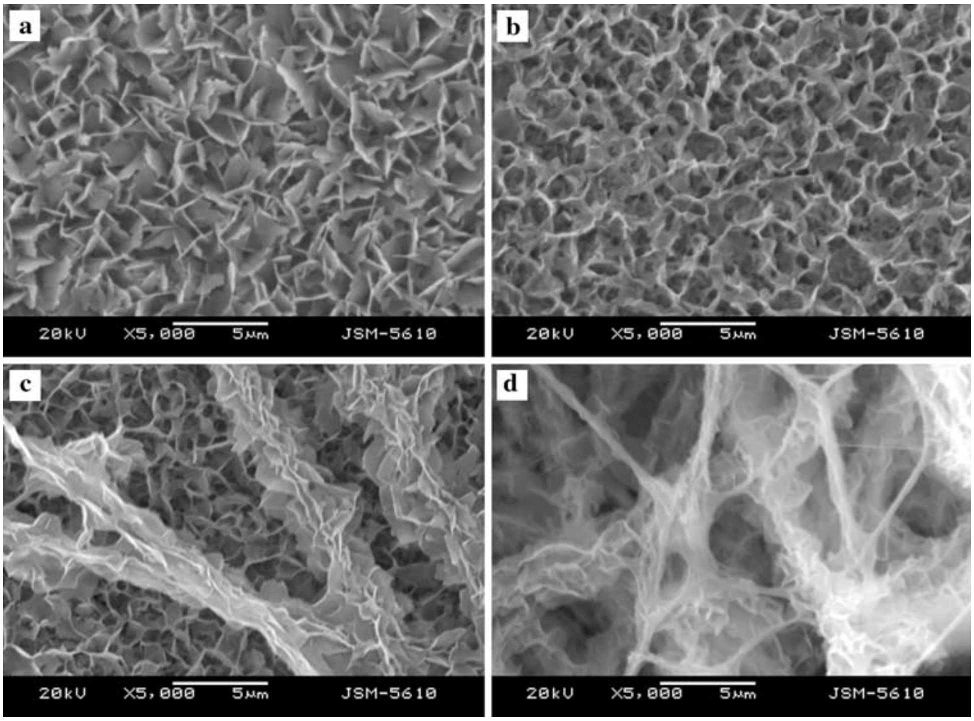

Fig. 1 Scanning electron micrographs of the coatings with different collagen concentrations: (**a**) coating without collagen, (**b**) 0.1 mg/ml, (**c**) 0.3 mg/ml, (**d**) 0.5 mg/ml

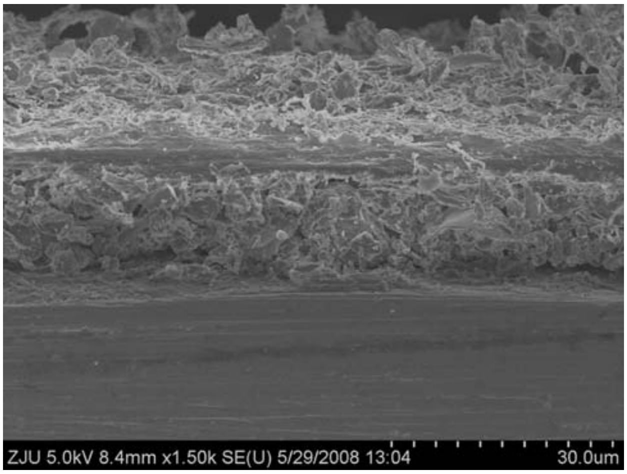

Fig. 2 Cross-sectional SEM photograph of the coating

homogeneous and well adheres to the substrate, and is about 20 µm in thickness.

A typical TEM image of the scratched chippings of the coating is shown in Fig. 3, in which flake-like Ca-P crystals can be clearly seen. The selected area diffraction of the coating chippings shows a ring pattern, which matches that of OCP phase [10]. The XRD pattern of the composite coating also shows the existence of OCP phase with the characteristic diffraction peak of (100) plane at 4.7°, as seen in Fig. 4.

The formation of the present OCP/collagen composite coatings involves the deposition of Ca-P crystals and collagen fibrils as a result of the cathode reaction. With regard to the deposition of Ca-P crystals, the cathode reaction on the Ti alloy substrate leads to the increase in pH around the electrode surface due to the generation of OH^- ions [11] as Eq. 1.

$$H_2O + 2e^- \rightarrow H_2(g) + 2OH^-, \tag{1}$$

Subsequently, the generated OH^- neutralizes $H_2PO_4^-$ to be HPO_4^{2-} and PO_4^{3-}. This triggers heterogeneous nucleation of Ca-P crystals near the cathode or alloy surface. In the present work, an OCP appears instead of HA during electrolytic deposition, it could be attributed to the deposition at low pH value [12]. The OCP formation is suggested by the following reactions:

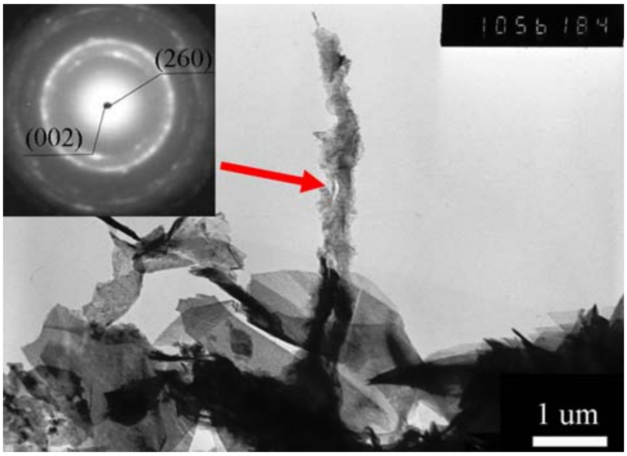

Fig. 3 TEM image of the minerals scratched from the composite coating. Inserted image is the selected area electron diffraction pattern

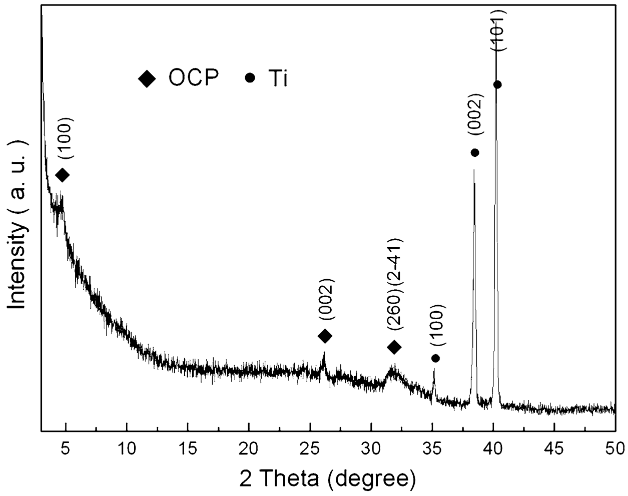

Fig. 4 XRD pattern of the calcium phosphate/collagen composite coating

$$OH^- + H_2PO_4^- \rightarrow HPO_4^{2-} + H_2O, \tag{2}$$

$$OH^- + HPO_4^{2-} \rightarrow PO_4^{3-} + H_2O, \tag{3}$$

$$4Ca^{2+} + HPO_4^{2-} + PO_4^3 = Ca_4(HPO_4)(PO_4)_2(OCP). \tag{4}$$

Since the collagen fibrils in solutions with pH below 5.5 are positively charged [13], the collagen fibrils are driven toward the cathode and enriched around during electrolytic deposition. The collagen fibrils could further serve as a heterogeneous nucleation reagent for Ca-P deposition because the terminal groups of collagen fibrils are mostly negative $-COO^-$ that might adsorb Ca^{2+} [14]. Hence, there could be two ways for the composite coating formation. One way is the deposition of collagen fibrils on the pre-formed OCP layer to become mineralized collagen fibrils later, and another way is the direct deposition of the mineralized collagen fibrils which are already formed in the electrolyte. The two ways are believed to occur simultaneously, and the former way may become dominant with increasing collagen concentration in the electrolyte because the naked fibrils are observed (Fig. 1d).

The result of the simple tape test showed that most of the collagen fibrils and a small amount of white powder were transferred to the tape, whereas the bulk of the Ca-P coating and some mineralized collagen fibrils remained on the substrate, as seen in Fig. 5. This suggests such composite coating is mechanically strong and well bonded to the Ti6Al4V substrate.

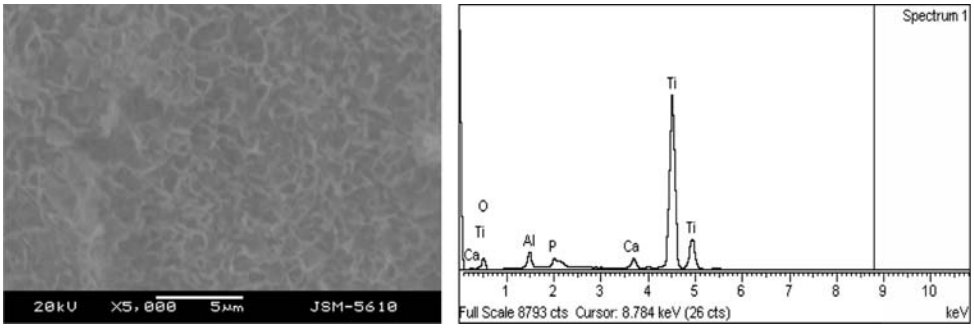

Fig. 5 Scanning electron micrographs of the coating after the simple tape test

4 Conclusion

In this study, homogeneous OCP/collagen composite coatings were successfully synthesized on Ti6Al4V substrates using electrolytic deposition method. The cathode reaction induced the deposition of OCP and drove collagen fibrils in the electrolyte moving toward the cathode or the substrate. The fibrils were mineralized through the deposition of OCP crystals, and the composite coating with porous structure formed on the substrates. This offers the possibility of fabricating desired surface matrix on orthopedic implant to enhance bone formation and fixation of orthopedic implants.

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References

1. B.D. Boyan, T.W. Hummert, D.D. Dean, Z. Schwartz, Biomaterials **17**, 137 (1996). doi:10.1016/0142-9612(96)85758-9
2. H. Assender, V. Bliznyuk, K. Porfyrakis, Science **297**, 973 (2002). doi:10.1126/science.1074955
3. M.P. Lynch, J.L. Stein, G.S. Stein, J.B. Lian, Exp. Cell. Res. **216**, 35 (1995). doi:10.1006/excr.1995.1005
4. U. Geißler, U. Hempel, C. Wolf, D. Scharnweber, H. Worch, K.W. Wengzel, J. Biomed. Mater. Res. **51**, 752 (2000). doi:10.1002/1097-4636(20000915)51:4<752::AID-JBM25>3.0.CO;2-7
5. S. Yashiki, R. Umegaki, M. Kino-Oka, M. Taya, J. Biosci. Bioeng. **92**, 385 (2001). doi:10.1263/jbb.92.385
6. Y. Honda, S. Kamakura, K. Sasaki, O. Suzuki, J. Biomed. Mater. Res. **80B**, 281 (2007). doi:10.1002/jbm.b.30595
7. C.V.M. Rodrigues, P. Serricella, A.B.R. Linhares, R.M. Guerdes, R. Borojevic, M.A. Rossi et al., Biomaterials **24**, 4997 (2003). doi:10.1016/S0142-9612(03)00410-1
8. S. Manara, F. Paolucci, B. Palazzo, M. Marcaccio, E. Foresti, G. Tosi et al., Inorg. Chim. Acta**361**, 1634 (2008). doi:10.1016/j.ica.2007.03.044
9. P. Peng, S. Kumar, N.H. Voelcker, E. Szili, R.S.C. Smart, H.J. Griesser, J. Biomed. Mater. Res. **76A**, 347 (2006). doi:10.1002/jbm.a.30514
10. Y. Fan, K. Duan, R. Wang, Biomaterials **26**, 1623 (2005). doi:10.1016/j.biomaterials.2004.06.019
11. S. Ban, S. Maruno, Jpn. J. Appl. Phys. **38**, 537 (1999). doi:10.1143/JJAP.38.L537
12. S. Ban, S. Maruno, Biomaterials **19**, 1245–1253 (1998). doi:10.1016/S0142-9612(98)00032-5
13. C.A. Farrugia, M.J. Groves, J. Pharm. Pharmacol. **51**, 643 (1999). doi:10.1211/0022357991772925
14. K. Hu, X. Yang, Y. Cai, Z. Cui, Q. Wei, Surf. Coat. Tech. **201**, 1902 (2006). doi:10.1016/j.surfcoat.2006.02.036