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Two step synthesis of CaP/TiO_2 nanorod composite coatings with improved protein adsorption

Kui Cheng [a,d,*], Tiantian Wang [a,d], Wenjian Weng [a,b,d,*], Mengfei Yu [c], Jun Lin [c], Huiming Wang [c], Piyi Du [a], Gaorong Han [a]

[a] Department of Materials Science and Engineering, State Key Laboratory of Silicon Materials, Zhejiang University, Hangzhou 310027, China
[b] The Shanghai Institute of Ceramics, Chinese Academy of Sciences, 1295 Dingxi Road, Shanghai 200050, China
[c] The First Affiliated Hospital of Medical College, Zhejiang University, Hangzhou 310003, China
[d] Cyrus Tang Center for Sensor Materials and Applications, Zhejiang University, Hangzhou 310027, China

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ABSTRACT

Titanium dioxide (TiO_2) nanorods and calcium phosphate (CaP) composite coatings were prepared and characterized. TiO_2 nanorod films were grown on titanium substrates via a hydrothermal method. Then, calcium phosphate was deposited into TiO_2 nanorod films electrochemically in an aqueous electrolyte containing calcium and phosphorus species. As a result, CaP/TiO_2 nanorod coatings were obtained. The amount of calcium phosphate deposited could be controlled through varying temperature, voltage and duration. It was found that both the presence of calcium phosphate and UV treatment could affect the surface wettability of the composite coatings. A bovine serum albumin adsorption test showed that the protein adsorption ability of the composite coatings was also improved with the presence of calcium phosphate and UV treatment. Moreover, the presence of calcium phosphate could promote the deposition of bone-like minerals. It is suggested that such coatings may exhibit promising initial cellular responses and thus be a good alternate for bioactive coatings.

1. Introduction

Titanium and titanium alloys have been widely used as implant materials because of good and reliable mechanical properties. However, they exhibit poor bioactivity [1] compared with bioactive materials such as titanium dioxide (TiO_2) or calcium phosphate (CaP). Therefore, titanium and its alloys coated with bioactive materials have attracted much attention in biomedical application [1,2] for many years.

As an important biomaterial, TiO_2 has been noticed for its good adhesion with titanium matrix, and good osseointegration with bone tissues. Nanoscale surface topography and the roughness of biomaterials have been recognized as crucial factors for tissue acceptance and cell survival [3]. Various nano-structured titania, such as nanodots, nanotubes and nanorods respectively, have been constructed on a titanium matrix to facilitate the integration of implants and host tissue. The nanorod structure was selected by many researchers due to its controllable diameter and density [4,5]. Moreover, the gap between the nanorods could be filled with a bioactive substance. Recently, some studies indicated that photo-induced superhydrophilicity of titania could even improve the proliferation and differentiation of bone marrow-derived osteoblasts, and eventually lead to improved osseointegration with bones [6–8].

Deposition of calcium phosphate on titanium implants has been investigated for many years. Several researches have demonstrated that thin calcium phosphate layers can effectively establish bonding between the implants and host bone tissue [9]. Such properties of calcium phosphate actually come from its osteoconductivity and similarity to bone mineral [1]. In addition, calcium ion (Ca^{2+}) sites on a titanium surface encourage protein adsorption, and influence cell proliferation, morphology and differentiation. This will facilitate the cell adhesion on PO_4^{3-} ion sites [2,10].

In view of all these considerations, surface coatings consisting of titanium dioxide and calcium phosphate may exhibit stronger performance. In this work, CaP/TiO_2-nanorod composite coatings were prepared through hydrothermal growth of TiO_2 nanorods and electrochemical deposition of calcium phosphate. The surface wettability, bovine serum albumin (BSA) adsorption property and in vitro simulated body fluid (SBF) mineralization property, as well as the effects of UV treatment on these properties, were characterized and discussed.

2. Materials and methods

2.1. Preparation of TiO_2 nanorod films on titanium substrates

TiO_2 nanorod films on titanium substrates were obtained by a hydrothermal synthesis process as described previously [5]. Briefly,

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* Corresponding authors at: Department of Materials Science and Engineering, State Key Laboratory of Silicon Materials, Zhejiang University, Hangzhou 310027, China. Tel./fax: +86 571 87953787.
E-mail addresses: chengkui@zju.edu.cn (K. Cheng), wengwj@zju.edu.cn (W. Weng).

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0.29 g picric acid was dissolved in 9.6 ml ethanol, and then was mixed with 36 ml deionized water, 24 ml concentrated hydrochloric acid (36.5%–38% by weight) and 0.17 ml tetra-n-butyl titanate. The mixture solution was served as a mother solution for the growth of TiO_2 nanorod films. Titanium foils (99.99% purity) with a thickness of 0.1 mm and 1.0 × 2.0 cm in dimension were placed into an autoclave with the mother solution and heated for 3 h at 160 °C for TiO_2 nanorod growth. After cooling down to ambient temperature, the Ti substrates were taken out, rinsed with deionized water and ethanol and allowed to dry in ambient air.

2.2. Preparation of CaP/TiO_2 nanorod composite coatings

The deposition of calcium phosphate on TiO_2 nanorod films was performed electrochemically through two-electrode modes, with a platinum foil serving as the counter electrode [11]. The electrolyte contained 4×10^{-3} M $Ca(NO_3)_2$ and 4×10^{-3} M $NH_4H_2PO_4$. A constant voltage of 3 V was used to deposit calcium phosphate at 50 °C for different time.

2.3. Surface characterization

The surface morphology and cross-section morphology of the coating were observed by a field emission scanning electron microscopy (FESEM HITACHI, S-4800) with the field emission electron source running at 3 kV. In order to observe the cross-section, the samples were cut off with scissors so that the cross-section could be exposed. The element composition was detected by energy dispersive X-ray detector (EDX) with a working voltage of 20 kV and a working current ranging from 3 mA to 10 mA. An X-ray diffractometer (XRD) and Fourier transform infrared (FTIR, NICOLET 830) spectroscopy were used to investigate the crystalline phase and chemical groups of the coatings. XRD patterns were recorded in a diffractometer (X'Pert PRO, PANalytical with Cu Kα radiation, $\lambda = 1.5406$ Å) from 10° to 80° at a scanning speed of 1° min^{-1}. The X-ray tube voltage and current were set at 40 kV and 40 mA, respectively. The FTIR patterns were recorded from 400 cm^{-1} to 4000 cm^{-1} at a step of 1 cm^{-1}. Water contact measurements were carried out to estimate the wettability of the surface.

2.4. BSA adsorption

As a model protein, BSA was selected to test the adsorption behavior of the composite coatings. All experiments were conducted at 37 °C in 1 mg/ml BSA solution for different duration. Phosphate buffered saline (PBS, pH = 7.4) was used to prepare the BSA solution. SEM was used to observe the morphology of the coatings after the adsorption of BSA. The coatings were put into 3 ml 1% sodium dodecyl sulfate (SDS) solution in centrifuge tubes and shaken for 1 h to detach BSA adsorbed. The samples were then taken out and the protein concentrations in the supernatant SDS solutions were determined through detecting the optical density at 280 nm using a UV spectrophotometer [12].

2.5. SBF mineralization

CaP/TiO_2 nanorod composite coatings were soaked in simulated body fluid to evaluate their in vitro bioactivity. The SBF was prepared by dissolving NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 into distilled water, and buffered with tris-hydroxymethyl aminomethane and HCl to pH 7.4 at 37 °C [13,14]. The samples were soaked in SBF at 37 °C for one week and then removed from the fluid and washed twice with deionized water.

3. Results and discussion

3.1. Intrinsic properties of CaP/TiO_2 nanorod composite coatings

SEM images of TiO_2 nanorod coatings before and after the deposition of calcium phosphates on titanium are presented in Fig. 1. It was observed that the TiO_2 nanorod films consisted of many individual TiO_2 nanorods (Fig. 1a). Calcium phosphate deposition was clearly observed in the gaps among nanorods, and became increasingly greater with increased deposition time, while the length of nanorods

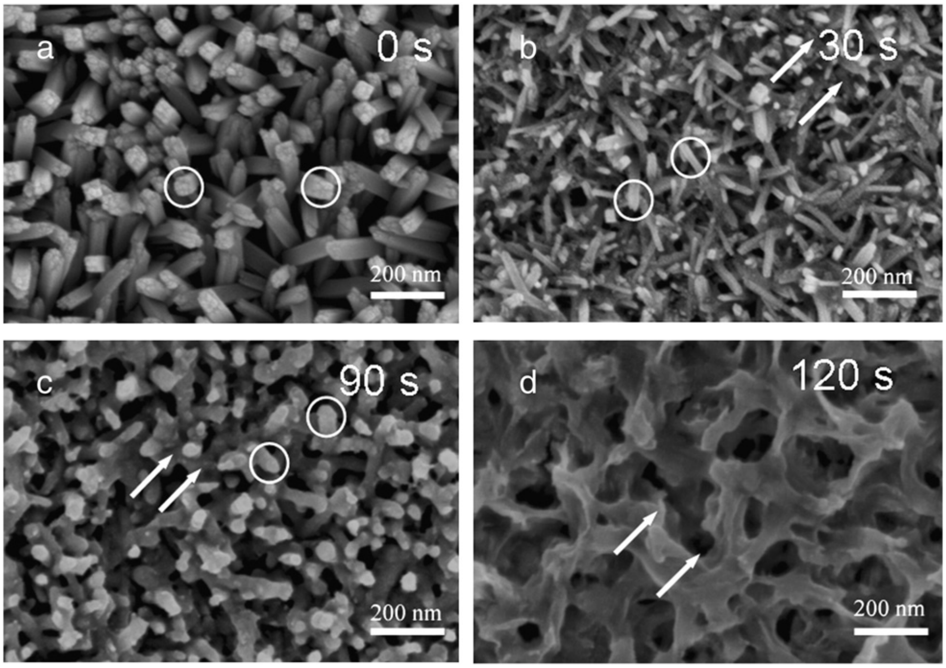

Fig. 1. Effects of calcium phosphate deposition on coating morphology. (a) Without CaP deposition; (b) 30 s CaP deposition; (c) 90 s CaP deposition; (d) 120 s CaP deposition. Circles indicate TiO_2 nanorods and arrows indicate calcium phosphate.