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Preparation of a beta-tricalcium phosphate nanocoating and its protein adsorption behaviour by quartz crystal microbalance with dissipation technique

Dongwen Pang, Liyong He, Luxi Wei, Huade Zheng, Chunlin Deng*

Guangdong Key Laboratory of Biomedical Engineering, School of Materials Science and Engineering, South China University of Technology, Guangzhou 510641, PR China

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ABSTRACT

Beta-tricalcium phosphate (β-TCP) nanocoatings, which can be analysed using a quartz crystal microbalance with dissipation technique (QCM-D), were fabricated on a gold surface by electrophoretic deposition. The influences of electric field intensity and electrophoresis time were investigated. The adsorption behaviours of bovine serum albumin (BSA) and lysozyme (LSZ) on Au and β-TCP surfaces were observed in real time by QCM-D. The homogeneous β-TCP nanocoating with moderately sized particles on gold surface was fabricated at 25 V/cm for 5 min, and it met the requirements for the QCM-D experiment. The adsorption behaviour of BSA was different from that of LSZ, which was caused by the differences of protein properties. The adsorption quantity of BSA on a β-TCP surface was higher than that on a gold surface. However, the adsorption amount of LSZ on a β-TCP surface was lower than that on a gold surface. The electrostatic force was the major factor affecting the adsorption quantities of BSA and LSZ on Au and β-TCP surfaces based on the investigation of various factors. The findings reported here will be useful for understanding the mechanism of the interaction between biomaterials and proteins.

1. Introduction

Beta-tricalcium phosphate (β-TCP), with excellent biocompatibility and biodegradability, has been extensively applied in orthopaedics as implant materials. In animal studies, β-TCP gradually resorbs and is replaced by remodelled bone [1,2]. Proteins in surrounding body fluids of implanted biomaterials are rapidly adsorbed on the surface [3,4]. The protein adsorption layer on the surface affects the subsequent interaction of cells with the implant material and the biomineralization process [5–7]. Therefore, it is necessary to study the adsorption behaviour of proteins on the implant material surface for biomaterial design.

The quartz crystal microbalance with dissipation (QCM-D) technique is a highly sensitive and practical tool for in situ measurements of mass changes in the range of nanograms as a frequency shift (Δf) and of viscoelastic property as a dissipation shift (ΔD) [8,9]. QCM-D has been widely applied to study the adsorption behaviours of proteins [10–12]. For example, a study of fibrinogen on a hydroxyapatite surface showed that the secondary structures change during the adsorption process, and the change is related to the adsorption amount of fibrinogen [10]. However, QCM-D has not been used to study the adsorption behaviours of proteins on a β-TCP surface. To comprehensively understand the interaction between proteins and the β-TCP surface, in situ monitoring the adsorption behaviours by QCM-D is necessary.

Many different substrates have been used in QCM-D, such as hydroxyapatite [13], metals [14], polymers [15], and functionalized coatings [16]. The β-TCP can be used in QCM-D by nanocoating it on a gold surface. There are many coating techniques, such as electrophoretic deposition (EPD) [17], plasma spraying [18], beam sputter deposition [19], and laser ablation [20]. EPD has the advantages of simple equipment, short formation time, and easy control of the deposition process [21]. Many studies reported that EPD has been extensively used in the preparation of hydroxyapatite sensors for QCM-D [10,22]. However, the preparation of β-TCP nanocoating by EPD for QCM-D has not been reported.

When the biomaterials are implanted into the body, different proteins in body fluids adhere to the substrate surface. Bovine serum albumin (BSA), with an isoelectric point of 4.7 and molecular weight of 66.5 kDa, has an asymmetric heart-like structure. At a pH of 7.4, BSA has a net negative charge because of the acidic amino acids on the side chains [13]. However, lysozyme (LSZ), with

* Corresponding author.
E-mail address: chldeng@163.com (C. Deng).

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an isoelectric point of 11.1 and molecular weight of 14.3 kDa, has a small compact globular structure. LSZ is net positively charged at a pH of 7.4 because of positively charged amino acids on the protein surface [23]. We chose these two proteins as model proteins to explore the adsorption behaviours on β-TCP surface because of the contrast effect between their properties.

The current study aims at investigating the adsorption behaviours of BSA and LSZ on β-TCP surface by QCM-D. We first used EPD to prepare the β-TCP nanocoating on a gold surface for QCM-D and optimized the preparation process. The β-TCP nanocrystals were prepared with a chemical precipitation. The effects of different protein types and substrate properties on the protein adsorption process were studied to deepen the understanding of protein adsorption.

2. Materials and methods

2.1. Synthesis of β-TCP

The β-TCP powders were synthesized using a chemical precipitation method. $Ca(NO_3)_2{\cdot}4H_2O$ and $(NH_4)_2HPO_4$, both analytical reagent grade, were used as sources of Ca and P, respectively. The major synthesis processes were as follows. Polyethylene glycol (PEG; 0.2 g, MW6000) was added as a dispersant to the $Ca(NO_3)_2$ (0.45 M) solution and stirred to ensure uniform mixing. The pH of the $(NH_4)_2HPO_4$ (0.5 M) solution was adjusted to 9.0 by adding ammonium hydroxide. The $(NH_4)_2HPO_4$ solution was added dropwise to the solution of $Ca(NO_3)_2$ at a rate of 5 mL/min under strong agitation. The pH of the reaction system was kept between 6.5–7.0 during the addition process. The mixture was stirred continuously for 10 h, then aged for 2 h at room temperature. The suspension was washed with deionized water until the pH of the supernatant was about 7.0. The resulting precipitate was frozen and dried, and then sintered at 800 °C for 3 h in a muffle furnace.

2.2. β-TCP sensors prepared by EPD

We used commercial gold-plated QCM-D sensors that were purchased from Q-Sense AB. The sensor surface was cleaned by immersing in a 5:1:1 mixture of Milli-Q quality distilled water, H_2O_2, and NH_3 for 5 min at 75 °C [11], and then blow-dried with nitrogen. The β-TCP nanocoating on the gold surface was deposited by EPD using a gold-plated sensor as the cathode and a platinum electrode as the anode. The electrolyte was 1 wt% of β-TCP suspension in ethanol. After applying 25 V/cm DC voltage for 1–10 min or 100 V/cm DC voltage for 1 min, the surplus β-TCP nanocrystals were removed by ultrasonic treatment for 1 min in ethanol.

The electrolyte was obtained after ball milling for 12 h. The electrical conductivity of the suspension was measured with an electrical conductivity meter (STARTER 3100C, USA) to characterize the stability of the suspension.

2.3. Real-time monitoring of protein adsorption by QCM-D

BSA and LSZ (chicken egg) were purchased from Sigma–Aldrich Company (Germany). The protein solution was prepared by dissolution in phosphate-buffered saline solution (GIBCO, pH 7.4) at the concentration of 1 g/L. The protein adsorption on Au and β-TCP surfaces was investigated using a QCM-D device (E4, Q-Sense AB, Sweden) at 5 MHz. The temperature was kept at 25.00 ± 1 °C. Phosphate-buffered saline was introduced into the sensor chamber to obtain a baseline. The protein solution was flowed through sensor surfaces at a rate of 40 μL/min, the time-dependent changes of the resonance frequency (Δf) and the energy dissipation (ΔD; overtone, n = 7) were recorded simultaneously.

2.4. Characterization

The phase composition of the β-TCP powders and the β-TCP nanocoating was resolved by X-ray diffraction (XRD; X'pert PRO, PANalytical, The Netherlands). Data were collected over a 2θ range of 10−70° with a step size of 0.02°. The morphology of β-TCP powders was observed by scanning electron microscopy (MERLIN Compact, Carl Zeiss, Germany). The surface topology, surface relative potential, and roughness of different substrates (Au and β-TCP) were analysed by atomic force microscopy (AFM; MFP-3D-S, Asylum Research, USA) using the taping mode. Surface wettability of different substrates was measured by a surface contact angle meter (OCA15, Data Physics, Germany) at room temperature.

3. Results and discussion

3.1. Analysis and characterization of the β-TCP powders

The XRD characteristic peaks of the β-TCP powders are shown in Fig. 1(a). The characterization peaks of the powders coincide with the characterization peaks of the standard card (JCPDS NO.090169 β-TCP). This result indicates that the synthesized powders were pure β-TCP phase without any other crystalline phase. The sharp diffraction peaks show that the synthesized powders are a high crystallinity. The morphology of the β-TCP powders determined by scanning electron microscopy is shown in Fig. 1(b). The β-TCP particles were near-spherical particles with different sizes on the nanometre scale.

3.2. Electrical conductivity measurement of the β-TCP suspension

The variation in electrical conductivity of the β-TCP suspension with time is shown in Fig. 2(a). After the suspension was aged for 6 h, the increase of electrical conductivity was slow and stable. The electrical conductivity of the β-TCP suspension represented the degree of protonation of β-TCP in ethanol. Therefore, a stable suspension was obtained after aging for 6 h.

3.3. Analysis and characterization of the β-TCP nanocoating on a gold surface

The XRD characteristic peaks of the β-TCP film on gold surface by EPD at 25 V/cm for 5 min are shown in Fig. 2 (b). In addition to the strongest peak of the gold, the rest of three strongest peaks coincide with the three strongest peaks of the standard card (JCPDS NO.090169 β-TCP). Therefore, the phase composition of the β-TCP powders did not change during the process of electrophoretic deposition. The XRD results of the β-TCP nanocoatings prepared under different conditions are basically the same.

Fig. 3(a–c) show AFM topographic images of gold and β-TCP films on gold surfaces prepared by EPD at 25 or 100 V/cm for 1 min. β-TCP powder particles were successfully deposited on the gold surface. Compared with the β-TCP coated surface, the gold surface was smoother and showed no obvious bumps. The root mean square (RMS) roughness of the gold surface was 0.50 ± 0.04 nm. The distinct sphere-like features were observed on β-TCP sensor surfaces, and they corresponded to the shape of β-TCP powder particles as shown in the microscopy image in Section 3.1. The effect of electric field intensity on the deposition process has been reported[24,25]. The high electric field intensity provides more power and higher mobility to nanoparticles; therefore, more nanoparticles with large sizes are deposited on a substrate surface. When the electric field intensity increased from 25 to 100 V/cm, there were more β-TCP nanoparticles with large sizes deposited on the gold surface. Therefore the RMS surface roughness increased