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Effect of phase composition and microstructure of calcium phosphate ceramic particles on protein adsorption

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ABSTRACT

The biological performance of biomaterials is strongly influenced by their protein adsorption characteristics, which are related to the structures and properties of both the biomaterial and the protein. In the present study two groups of hydroxyapatite (HA) and biphasic calcium phosphate (BCP) ceramic powders were fabricated by different drying processes. The roles of the phase composition and microstructure of the powders in the adsorption of various model proteins were evaluated. The experimental results showed that BCP always had a higher ability to adsorb fibrinogen, insulin or type I collagen (Col-I) than HA. The microporosity and micropore size of the CaP particles also had a strong impact on their protein adsorption characteristics. HA and BCP particles with higher microporosities and/or more micropores >20 nm in diameter could adsorb more fibrinogen or insulin. However, amounts of adsorbed Col-I were largely unaffected by the microstructure of HA and BCP particles.

1. Introduction

When biomaterials are in contact with a living body proteins from the surrounding body fluids will be spontaneously adsorbed onto their surfaces within seconds and modulate subsequent cell attachment, spreading and migration [1,2]. Thus, interactions between proteins and the material surface is an important determinant of the success of an implant. The protein adsorption behavior also plays a vital role in the fields of biosensor design [3] and drug delivery systems [4]. So far a large number of studies have reported that proteins such as fibronectin, bone morphogenetic proteins and so forth can enhance cell adhesion to the implant surface or even promote osteoblastic differentiation and bone regeneration [5–9]. However, the protein adsorption characteristic of biomaterials and the mechanisms involved need further investigation. An intensive knowledge of protein adsorption is not only beneficial to the optimization of the surface structure of biomaterials but also helpful in determining specific applications within the field of biomedical.

Among biomaterials used for hard tissue repair and substitution calcium phosphate-based (CaP) biomaterials have been widely used for decades due to their resemblance to the inorganic composition of bone and teeth. Their osteoconduction and even osteoinduction in vivo have been confirmed by many researches [10–14]. They have also been considered as delivery carriers for some growth factors and anti-cancer drugs [15–18]. Previous studies have shown that the bioactivity of CaP ceramics varies with phase composition, sintering temperature and surface structure [12,13,19,20]. These variances in biological performance might result from the adsorption characteristics for proteins. We have previously reported that the porous structure of a biphasic calcium phosphate (BCP) ceramic played an important role in the adsorption of serum proteins and it notably enhanced the adsorption of transforming growth factor β1 on the BCP surface [21]. Li et al. confirmed that microstructured CaP materials with increasing surface areas could concentrate more proteins, which might facilitate the differentiation of inducible cells into osteogenic cells [22]. Here we have tried to look for a correlation between the surface micro/nanostructure of CaP ceramics and protein adsorption and thus explore the possible effects of this micro/nanostructure on the biological performance of CaP ceramics.

In the present study two groups of hydroxyapatite (HA) and BCP ceramic powders with different microstructures were fabricated by controlling the drying process. Type I collagen (Col-I) is an extracellular matrix protein abundant in skin, bone and tendon [1,23]. Fibrinogen is a large plasma protein, and its adsorption plays a key role in blood clotting, as well as modulating subsequent acute and chronic inflammatory responses [24,25]. Insulin regulates the concentration of glucose in the blood of most mammals [26]. The above three proteins were selected as models to investigate the protein adsorption characteristics of the CaP powders, and the roles of phase composition and microstructure in protein adsorption are discussed in detail.

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2. Materials and methods

2.1. Material preparation

$Ca(NO_3)_2$, $(NH_4)_2HPO_4$ and NH_4OH of analytical grade were commercially available (Chengdu Kelong Chemical Reagent Plant, China) and were used without further purification. The wet precipitation method was used to produce HA and BCP precursors. For HA (or BCP) precursor 1000 ml of 1.0 M $Ca(NO_3)_2$ was added dropwise into 600 ml (or 617 ml) of 1.0 M $(NH_4)_2HPO_4$, pH 10.0 (adjusted by addition of NH_4OH). The pH of the reactive system was maintained at ~10.0 by dropwise addition of NH_4OH. The slurries obtained were aged at 80 °C for 24 h, washed with deionized water and then dried by two different processes. The Group I powders (HA I and BCP I) were obtained by pump filtering the slurries and drying the precipitates in an oven at 80 °C overnight before grinding in a mortar. The Group II powders (HA II and BCP II) were produced by spray drying the slurries with a SFDC-20 Spray Dryer (Shanghai Ohkawara Dryers Co., China). The collected powders of both groups were sintered at 1100 °C for 2 h and then crushed and sifted through a 120-mesh (~125 μm) sieve.

2.2. Material characterization

The phase compositions and morphologies of the four types of CaP powders were analyzed by X-ray diffraction (XRD) (X'Pert Pro, Philips, The Netherlands) and scanning electron microscopy (SEM) (JSM-5900 OL, JEOL, Japan), respectively. The particle size distributions, specific surface areas (SSAs) and microporosities of the HA and BCP powders were measured with a Laser Diffraction Particle Size Analyzer (SALD-2100, Shimadu, Japan) and Surface Area and Pore Size Analyzer (SA 3100, Beckman-Coulter, USA), respectively. The SSA was determined by N_2 adsorption according to the BET method and the micropore volume and size were analyzed using the BJH method.

2.3. Protein adsorption study

Fibrinogen, insulin and Col-I (Sigma, America) were used as model proteins. The protein solutions were prepared by dissolution in phosphate-buffered saline (PBS), pH 7.4, and the protein concentrations were measured with a BCA™ Protein Assay Kit (Pierce, USA).

Protein adsorption experiments were carried out in 1.5 ml low adsorption microcentrifuge tubes. Samples of CaP ceramic powders (0.1 g) were equilibrated with 0.9 ml of PBS for 1 h at 37 °C, then 0.1 ml of protein solution, at a concentration of 1.0–10.0 mg ml^{-1} was added to the tube. After incubation at 37 °C for 2 h with continuous shaking the mixtures were centrifuged for 5 min at 10,000 rpm and the supernatants were quantitatively assayed by the BCA method using absorbance values at a wavelength of 570 nm against a PBS blank, determined in a Model 550 Microplate Reader (Bio-Rad, USA). The amounts of adsorbed protein on the particles were calculated from the mass balance. All experiments were carried out in triplicate.

3. Results

3.1. XRD analysis

Fig. 1 shows the XRD patterns of the four types of CaP powders. Obviously, the two drying processes did not influence the phase composition of either the HA or BCP powders. HA I and HA II had the same XRD patterns and were both composed of pure HA. Similarly, both BCP I and BCP II consisted of HA and β-tricalcium phosphate (β-TCP) phases. The HA/β-TCP mass ratio in BCP was about 70/30, analyzed according to ISO-13779-3 [27]. Simply, based on the intensities of the characteristic peaks of HA and β-TCP observed, respectively, at $2\theta = 31.81°$ and 31.07°, the HA/β-TCP mass ratio could be obtained by calculating the ratio of the integrated intensity of peak (2 1 1) of HA to the integrated intensity of peak (0 2 10) of β-TCP and referring to a calibration curve.

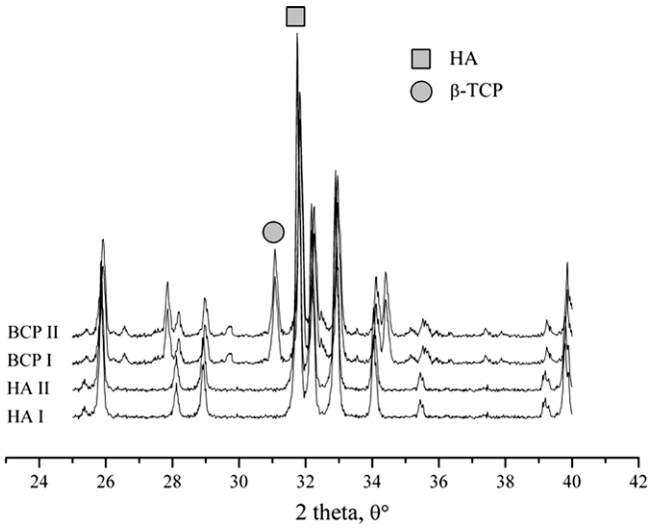

Fig. 1. XRD patterns of the four types of CaP powders.

3.2. SEM observations

Fig. 2 shows typical SEM micrographs of the four types of CaP powder. The Group I powders (HA I and BCP I) consisted of irregularly shaped particles with a wide size distribution. However, Group II powders (HA II and BCP II) had a regular ball-like shape and the particles had a relatively narrow size distribution. This indicated that the drying process influenced the shape and size of CaP particles. On the other hand, the effects of the drying process on the microstructures of HA and BCP particles were different. HA I had more micropores than HA II, while BCP I had a similar microporous structure to BCP II. The phase compositions had a greater impact on the microstructure of Group II powders than those of Group I. HA II had fewer micropores than BCP II, but there was no obvious difference in microstructure between HA I and BCP I.

3.3. Particle size analysis

The results of the particle size analysis of the four types of CaP powders are shown in Fig. 3. Consistent with the SEM observations, the HA I and BCP I particles had similar wide size distributions, which ranged from several hundred nanometers to several hundred micrometers. However, Both HA II and BCP II showed narrow ones, and the particle diameters were mostly around 50 μm.

3.4. SSA and micropore analysis

The SSAs and microporosities of the four types of CaP powders are summarized in Table 1. The data show that the four types of CaP powders produced no significant differences in SSA. On the other hand, the microporosities of CaP powders were influenced by their phase compositions and the drying process. For both Group I and Group II BCP powders had higher microporosities than HA powders. However, the effects of the drying process on the microporosities of HA and BCP particles were different. HA I had

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