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Kinetics and thermodynamics studies on the BMP-2 adsorption onto hydroxyapatite surface with different multi-morphological features

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ABSTRACT

The effect of the surface topography on protein adsorption process is of great significance for designing hydroxyapatite (HA) ceramic material surfaces. In this work, three different topographies of HA materials HA-sheet, HA-rod, and HA-whisker were synthesized and testified by X-ray diffraction (XRD), Fourier transform infrared (FT-IR), Brunauer–Emmett–Teller (BET) and a field emission scanning electron microscopy (FE-SEM). We have systematically investigated the adsorption kinetics and thermodynamics of bone morphogenetic proteins (BMP-2) on the three different topography surfaces of HA, respectively. The results showed that the maximum adsorption capacities of HA-sheet, HA-rod and HA-whisker were (219.96 ± 10.18), (247.13 ± 12.35), and (354.67 ± 17.73) $\mu g \cdot g^{-1}$, respectively. Kinetic parameters, rate constants, equilibrium adsorption capacities and related correlation coefficients, for each kinetic model were calculated as well as discussed. It demonstrated that the adsorption of BMP-2 onto HA could be described by the pseudo second-order equation. Adsorption of BMP-2 onto HA followed the Langmuir isotherm. It confirmed that compared with other samples HA-whisker had more adsorption sites for its high specific surface area which could provide more opportunities for protein molecules. The adsorption processes were endothermic ($\Delta H > 0$), spontaneous ($\Delta G < 0$) and entropy increasing ($\Delta S > 0$). A possible adsorption mechanism has been proposed. In addition, the BMP-2 could be adsorbed to the surface which existed slight conformational changes by FT-IR.

1. Introduction

Hydroxyapatite [HA, $Ca_{10}(PO_4)_6(OH)_2$] is the most potential implant biomaterial because it is osteoconductive [1–3] and has excellent biological affinity with bony tissue, possessing a similar chemical composition and structure to the mineral phase of bones [4]. HA has also been investigated as a scaffold of bone morphogenetic proteins (BMPs) for bone regeneration [5–7]. BMPs belong to the transforming growth factor-β super family [8]. Only a small amount of BMPs exists in postnatal life and plays a role in bone regeneration after bone injury such as bone fracture, especially bone morphogenetic protein-2 (BMP-2) [9]. BMP-2 has often been combined with HA materials by dropping the BMP-2 solution onto a HA molding form. In this case, the interactions between HA and BMP-2 might be observed. An increasing number of studies have investigated the process of BMP-2 adsorption on HA [10–15], and the previous studies mainly focused on the adsorption characteristics of BMP-2 on HA. T. Boxi et al. [10] studied the adsorption isotherms and determined that there is a strong affinity between BMP-2 and HA surface. And the interactions between the functional groups from the protein and calcium ions from the HA surface (the (001) HA plane) came out of Xiuli Dong's [15] study based on theoretical modeling. The process of BMP-2 adsorption on HA is influenced by many factors, such as the specific structural characteristics of the protein [10,16–18] and the surface properties of the HA [19–21]. The reviewed literature introduced that both structure and composition of CaP ceramics affected the protein adsorption behaviors [22]. However, there have been few studies on multi-morphological HA adsorbed BMP-2.

In this study, the three topographies of HA sheet-like (HA-sheet), rod-like (HA-rod), and whisker-like (HA-whisker) were synthesized by different methods. The BMP-2 protein adsorption kinetics [10,23] and thermodynamics on the different surface topographies of HA were analyzed to reveal the mechanism of the protein adsorption and the effects of the topographies on protein adsorption. It is a challenge for the experiments to provide enough microscopic details about how the BMP-2 interacts with the HA. An intensive knowledge of the process of BMP-2 adsorption on the HA is not only beneficial to the optimization of biomaterials' surface structure, but also helpful to develop specific applications within the field of biomedicine.

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2. Materials and methods

2.1. Materials and reagents

Calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$, ≥99.0%), phosphoric acid (H_3PO_4, ≥85%), Nitric acid (HNO_3, ≥68%), acetamide (C_2H_5NO, ≥99.0%), hexadecyltrimethylammonium bromide (CTAB), Citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$, ≥99.5%), ammonia solution (25%), ammonium phosphate dibasic ($(NH_4)_2HPO_4$, ≥99.0%), dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$, ≥99.0%) and ethanol (CH_3CH_2OH, ≥99.7%) were purchased from KeLong (China). Phosphate saline buffer (PBS) at pH 7.4 was purchased from Boster (China). Proteins: BMP-2 was purchased from Shanghai Rebone Biomaterials Co. (China). Enhanced BCA Protein Assay Kit was purchased from the Beyotime Institute of Biotechnology (China). All the chemicals were used as purchased, without further purification. All the aqueous solutions were prepared with distilled water.

2.2. Material preparation

2.2.1. HA-sheet

Analytical-grade chemicals $(NH_4)_2HPO_4$, $Ca(NO_3)_2 \cdot 4H_2O$, HNO_3 and acetamide were used as received. $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ were dissolved in 0.05 mol · L^{-1} HNO_3 solution, with the calcium to phosphorus molar ratio of 1.67. Then 0.5 mol · L^{-1} acetamide solution was dissolved in the mixed solvent above. The pH was adjusted to 3.3 by 0.1 mol · L^{-1} HNO_3. The prepared solution was heated to 180 °C and then maintained for 15 h. The mixture was cooled naturally to room temperature (about 25 °C). The obtained white precipitate was collected and washed with distilled water several times, and then dried in air at 80 °C [24].

2.2.2. HA-whiskers

$Ca(NO_3)_2 \cdot 4H_2O$, $(NH_4)_2HPO_4$ and urea were dissolved in 25 mL distilled water, with the calcium to phosphorus molar ratio of 1.67. The pH was adjusted to the 3.2 by 0.5 mol · L^{-1} HNO_3. Then, the as-obtained mixing solution was transferred into a Teflon bottle held in a stainless steel autoclave, sealed, and made the temperature cycle (70 °C–90 °C) during the experimental procedure. The temperature increasing and decreasing step at a speed of 0.5 °C · min^{-1}. Firstly, we increased the temperature to 90 °C and kept it for 12 h. Secondly, we made the temperature decrease to 70 °C [25]. And we repeated the procedures above six times. After, the mixture was cooled naturally to room temperature (about 25 °C). The obtained white precipitate was collected and washed with distilled water several times, then dried in air at 80 °C.

2.2.3. HA-Rod

Aqueous solutions of $Ca(NO_3)_2 \cdot 4H_2O$ (1 mol · L^{-1}) and H_3PO_4 (0.6 mol · L^{-1}) were added into a three neck flask kept in a 40 °C water bath, with the calcium to phosphorus molar ratio of 1.67. Then an aqueous solution containing citric acid [26] as organic modifier (5% theoretically produced HA) was added into the mixed solution under stirring. The pH was adjusted to 10 by adding ammonia solution (25%). After reacting at 40 °C for 4 h, the reaction solution was transferred into a Teflon-lined autoclave and autoclaved at 200 °C for 8 h. After, the mixture was cooled naturally to room temperature (about 25 °C). The obtained white precipitate was collected and washed with distilled water several times, and then dried in air at 80 °C [26].

2.3. Sample characterization

The phase compositions of the three topographies of HA ceramic were analyzed by Powder X-ray diffraction (XRD). XRD data were collected on a D/Max-RA diffractometer (DX-2600, Dan-Dong China) with Cu Kα-radiation (λ = 0.1548 nm) operated at 40 kV and 100 Ma.

The morphological features of the three topographies of HA ceramic were observed by using scanning electron microscopy (SEM, JEOL JSM 7500 F). The specific surface area (SSA) was measured by N_2 adsorption according to the BET method using QUADRASORB SI automated surface area and pore size analyzer (Quantachrome Instruments, USA). The Fourier transform infrared spectra of the samples were performed by the KBr pellet technique on a Fourier transform infrared spectrometer (IR Nicolet PerkinElmer Co.).

2.4. Protein adsorption

2.4.1. Adsorption kinetics

Adsorption experiments investigated the interaction of different topographies of HA for BMP-2. The protein solution was prepared by dissolving the BMP-2 powder in PBS solution and the concentration of the BMP-2 solution was 3.5 μg · mL^{-1}. The weight of the HA was 0.1 g. Each HA sample was placed in the polypropylene centrifuge tubes, then 200 mL BMP-2 solution was added into the tubes. The HA ceramic particles were suspended in a protein solution. The suspension was stirred continuously at 25 °C, then the supernatant was taken out at regular time and centrifuged at 8000 rpm. After centrifugation the supernatant was filtered through a membrane filter to remove the rest of HA. The BMP-2 concentration in the supernatant was determined by using the BCA Protein Assay Kit (Beyotime). The absorbance of the sample was measured by the microplate reader (Model 550, Bio-Rad) at the maximum absorbance wavelength of 570 nm with a phosphate buffer (pH = 7.4) as a baseline.The amount of BMP-2 adsorbed at equilibrium 'q_e' (μg · g^{-1}) on HA ceramic particles was defined as follows in Eq. (1)

$$q_e = (C_0 - C_t)\frac{V}{W} \tag{1}$$

where C_0 is the initial concentration of BMP-2, C_t is the concentration of a certain time t, V is the reaction solution volume, and W is the mass of HA ceramic particles.

2.4.2. The model used for protein adsorption

In order to examine the controlling mechanism of the adsorption process and obtain characteristic constants of adsorption. The models of pseudo-first-order equation and pseudo-second-order equation were used to test the experimental data.

The pseudo-first-order model is generally expressed as follows in Eq. (2):

$$\frac{dq_t}{d_t} = k_1(q_e - q_t). \tag{2}$$

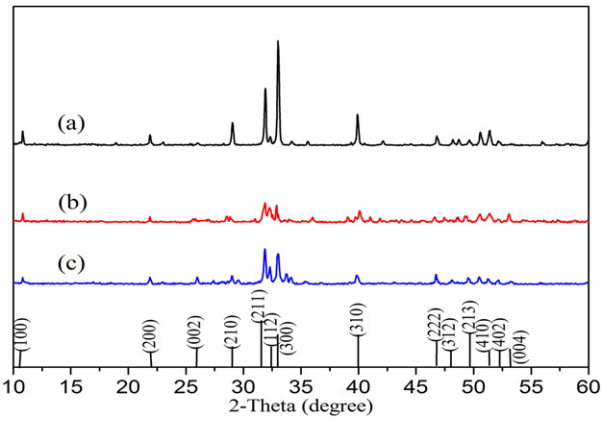

Fig. 1. X-ray diffraction of the HA: (a) sheet, (b) rod, and whisker.