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ScienceDirect
Acta Biomaterialia 5 (2009) 1311–1318
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Effect of surface structure on protein adsorption to biphasic calcium-phosphate ceramics in vitro and in vivo

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Received 25 April 2008; received in revised form 9 October 2008; accepted 25 November 2008
Available online 10 December 2008

Abstract

Protein adsorption affects the function of cells and determines the bioactivity of biomaterial implants. Surface structure and properties of materials determine the behavior of protein adsorption. In the present study, two biphasic calcium-phosphate ceramics (BCPs) with different surface structures were fabricated by pressing and H_2O_2 foaming methods. Their surface characteristics were analyzed and the in vitro and in vivo protein adsorption on them was investigated. Porous BCP showed higher ability to adsorb proteins, and transforming growth factor-β1 (TGF-β1) adsorption notably increased with increasing in vivo implantation time. The strong affinity of BCP to TGF-β1 might provide important information for exploring the mechanism of the osteoinduction of calcium phosphates.

Keywords: BCPs; Surface structure; Protein adsorption; TGF-β1

1. Introduction

The bioactivity of biomaterials is one of the factors that determine the successes of the implants. It is known that the initial event is the adsorption of various proteins from blood or other body fluids to the biomaterial after implantation [1–3], and the adsorbed proteins would affect the behavior of cells and then determine the bioactivity of the implant [4–6]. However, protein adsorption is a complex process and is dependent on the surface properties of a biomaterial, such as the chemical composition, structure, surface charge and so on [7]. Therefore, the full knowledge for the interaction between proteins and the biomaterial surface is helpful for us to understand and reveal the biological nature of the implant.

Calcium phosphates have a similar chemical composition to the inorganic phase of human bone tissue and have been extensively studied for a long time in the biomaterials field. Since the osteoinduction of calcium phosphates was reported in 1991 [8,9], biomaterials scientists have been exploring its mechanism, which is not yet completely clear. It had been confirmed that the appropriate porous structure is necessary for the osteoinduction of calcium-phosphate ceramic [10–12] and the dense one could not induce bone formation [13,14]. Moreover, BCP consisting of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) has better osteoinduction than single phasic HA or β-TCP [15,16]. It is known that various bone growth factors, especially the super family of transform growth factors including bone morphogenetic protein-2 (BMP-2) and TGF-β1, would participate in the process of bone formation and remodeling [17]. Yuan et al. reported that calcium-phosphate ceramic loaded with BMP-2 showed enhanced osteoinduction [18,19]. Zhang et al. believed that the ability to concentrate various bone growth factors under physiological conditions should be necessary for the osteoinduction of calcium phosphates [16].

We previously reported that various calcium phosphates had similar protein adsorption behavior, and a basic

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doi:10.1016/j.actbio.2008.11.024

lysozyme with lower molecular weight showed higher adsorption affinity for calcium phosphates than the acidic bovine serum albumin [20]. In fact, biomaterials are usually exposed to human plasma or other body fluids that contain hundreds and thousands of proteins, and a dynamic protein exchange will be observed when these proteins simultaneously adhere to the surface after implantation of a biomaterial, so the preferential adsorption of some bone-related proteins, especially bone growth factors, on calcium phosphates might be related to its osteoinduction. So far, there are many documents dealing with behaviors of in vitro protein adsorption on biomaterials [21–27]. However, in vivo studies have rarely been done. As a valuable in vivo research tool for the study of immunology, tissue growth and organ transplants, diffusion chambers have been widely used for biomaterials research [28–30]. Diffusion chambers can inhibit cells and permit various ions, organic small molecules and proteins to enter into it, so the interaction of biomaterials with proteins in body fluids, not the influences of cells, can be investigated. In the present study, we make two BCP ceramics with different surface structures and investigate the adsorption behavior of serum proteins, especially TGF-β1 on them in vitro and in vivo. The effect of surface structure on protein adsorption is discussed in detail.

2. Materials and methods

2.1. BCP ceramics

BCP precursor powders (HA/TCP: 70/30) prepared by a wet precipitation method were supplied by National Engineering Research Center for Biomaterials of China. Two BCP ceramics with different structures were prepared, respectively, by pressing and H_2O_2 foaming methods. The relatively dense ones (DBCP) were fabricated by placing the precursor powders into a special mold mounted in a single-axial press and pressing it under 300 MPa for 5 min. The porous ones (PBCP) were made by foaming BCP precursor powders with 5% solution of H_2O_2. After being sintered at 1100 °C, the starting DBCP disks were polished with 1200-grit sandpaper and then 1 μm of diamond powders, and the PBCP cylinders were cut into disks and then polished with 1200-grit sandpaper. The obtained DBCP and PBCP disks were cleaned by a sonic cleaner with ethanol and dd-H_2O, and then dried at 80 °C in an oven. Prior to use, all samples were autoclaved at 120 °C for 30 min.

2.2. Rat serum

The blood samples, which were collected from healthy adult SD rats (200 ± 20 g, supplied by Experimental Animal Center, Sichuan Institute of Chinese Materia Medica) clotted for 2 h at room temperature before centrifuging for 20 min at approximately 2000 rpm. The serum was removed and stored at $\leqslant -20$ °C before being used for adsorption experiments.

2.3. Materials characterization

The phasic compositions of the two BCP ceramics were measured with X-ray diffraction (XRD, Philips X'Pert Pro MPD). The morphologies of them were observed by scanning electron microscopy (SEM, JSM-5900 OL). The specific surface areas of them were measured by nitrogen adsorption according to the BET method.

Zeta potentials of the two BCP ceramics were performed using SurPASS apparatus (Anton Paar, Austria). The streaming currents were measured with the SurPASS using the Adjustable Gap Cell on which the samples with 20 mm × 10 mm × 1 mm size were mounted in the presence of a 10^{-3} M solution of KCl, and the zeta potentials were evaluated from streaming current measurements according to

$$\zeta = \frac{dI}{dp} \times \frac{\eta}{\epsilon \times \epsilon_0} \times \frac{L}{W \times H} \quad (1)$$

where ζ is the zeta potential; $\frac{dI}{dp}$ is the slope of streaming current vs. different pressure; η is the electrolyte viscosity; ε is the permittivity; ε_0 is the dielectric coefficient of electrolyte; and L, W, H are the length, width and height of the streaming channel.

2.4. Protein adsorption experiments in vitro and in vivo

In vitro experiments were done by incubating the DBCP or PBCP disks in 5 ml of capacity polypropylene centrifuge tubes with 2 ml of rat serum at 37 °C and 5% CO_2 for 3 days. As for in vivo experiments, the samples were put inside the diffusion chambers (Millipore Co., USA), which are Plexiglas® rings 14 mm in diameter with MF-Millipore Membrane filters having 0.45 μm of pore sizes glued to the top and bottom rims of the rings, and then implanted into the dorsal muscle of the adult SD rats under general anesthesia and sterile conditions. After 3 and 7 days of implantation, the diffusion chambers were retrieved and the samples were removed. All samples were transferred into new tubes and washed three times with dd-H_2O to remove any loose-binding proteins, then the proteins adhering to the sample surface were desorbed by 200 μl of 0.5 M solution of Na_2HPO_4 with sharp stirring for 15 min. The supernatants containing the desorbed proteins from the materials' surface were collected and diluted until the Na_2HPO_4 concentration lowered to 0.1 M for the next analysis. Changes to the surface morphology and compositions of the materials were examined using XRD and SEM, respectively.

2.5. Polyacrylamide gel electrophoresis (PAGE) analysis

The eluted protein samples (50 μl out of 300 μl in total) were subjected to separated by SDS–PAGE, which was performed through 5–15% gradient gels in a PROTEAN® II xi electrophoresis system (Bio-Rad, USA) according to the method of Laemmli [31]. The gels were stained with Coomassie brilliant blue R-250 and scanned with Chemi-Doc™ XRS Systems (Bio-Rad, USA). The protein bands

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