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Original Article

Biocompatibility and Biodegradation of Multiphasic Calcium Phosphate Ceramic Bone Substitute Transformed by Ostrich Cancellous Bone for Bone Tissue Engineering

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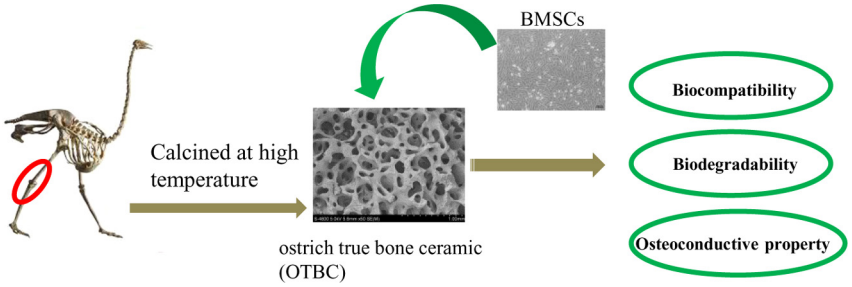
Graphical abstract



Abstract

A kind of novel multiphasic calcium phosphate ceramic bone substitute transformed by ostrich cancellous bone (OCB) was explored in this article. In this study we investigated the characterization, biocompatibility, biodegradability and in vivo osteoconductive property of the material. Scanning electron microscope (SEM) and X-ray diffraction (XRD) were used to characterize the material. Biocompatibility and biodegradation were confirmed by testing the hemolysis and intramuscular implantation. Bone marrow stromal cells (BMSCs) were also used to perform biocompatibility. Besides, material/BMSCs composites were implanted into the subcutaneous of back of nude mice to evaluate the osteoconductive property of the material. SEM analysis showed that the presence of a large amount of micropores within macropore walls and the interporous connections were abundant which indicated that the material possessed enough surface area. XRD pattern showed the peaks were corresponding to the characteristic for hydroxyapatite (HA), β-tricalcium phosphate (β-TCP), and sodium calcium phosphate ($NaCaPO_4$). Culturing of BMSCs on the scaffolds revealed the cells were able to attach and proliferate easily on the surface and the pores of the scaffolds. Moreover, this scaffolds had a good performance of biodegradation in vivo. Finally, histological observation of heterotopic bone formation revealed newly formed bone at 8 weeks after transplantation. The results of this study demonstrated that the multiphasic calcium phosphate ceramic had good biocompatibility and its surface configuration and pore structure were suitable for BMSCs to attach and proliferate and also had a good osteoconductive property, which suggested that the material could be used as a bone tissue engineering scaffold in the future.

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1. Introduction

Large amounts of bone graft are frequently used to reconstruct the maxillofacial bone defects resulting from tumor resection, trauma, and infection. Autograft and allograft bone are often used, but each has its own limitations [1,2]. To avoid the potential risks, many artificial bone substitutes, including bioceramics, polymers, alloys, and composite biomaterials have been studied [3–5]. Among them, calcium phosphate ceramics, e.g. hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP) have been extensively studied due to their good biocompatible and osteoconductive properties [6,7].

Sintered bovine bone, or true bone ceramic (TBC) has been used as a substitute for bone formation for many years and also have achieved some satisfactory results [8]. TBC is a porous biomaterial with a natural trabecular structure like that of cancellous bone and is composed of inorganic bone material. Because it is formed by sintering at a high temperature, TBC is not immunogenic, and no cytotoxicity is observed [9]. Augmentation of the natural osteoconductivity of TBC with osteoinductive agents such as bone morphogenetic proteins (BMPs), bone marrow, and osteoblasts showed good cell compatibility and yielded promising osteogenic properties [9,10]. Ostrich cancellous bone (OCB) is a kind of new xenogenic biomaterial which is rarely mentioned by other literatures. In our previous study, we have demonstrated that sintered OCB could be used in bone reconstruction, but their characterizations need to be further improved [11].

In our experiments, OCB was first given a chemical management, followed by calcined in a high temperature and then was transformed to multiphasic calcium phosphate ceramic through another chemical management. This material was named ostrich true bone ceramic (OTBC). The purpose of this study was to observe the characterization, biocompatibility, biodegradation, and osteoconductive property of OTBC in vivo and to discuss whether it is suitable for using as scaffold material in bone tissue engineering.

2. Materials and methods

2.1. Ethics statement

This study was reviewed and approved by the Institutional Animal Care and Use Committee at the Fourth Military Medical University, Xi'an, China. Animals were cared for according to established institutional guidelines, and all efforts were made to minimize suffering. New Zealand rabbits (4 months old, with an average weight of 2.5 kg) and severe combined immunodeficiency (SCID) mice (6 weeks old, weighing 20–25 g) were originally obtained from the animal center of the Fourth Military Medical University.

2.2. Preparation and characterization of ostrich true bone ceramic (OTBC) scaffolds

OTBC scaffolds were fabricated by physical and chemical methods, including three stages, as follows: Stage1. The original ostrich cancellous bone (OCB) materials were obtained from proximal part of tibial bone and cut into cubes of approximately $2 \times 1 \times 0.5$ cm^3. Soft tissues such as muscle and tendons were removed from the bone. The pieces were immersed in 2% sodium hydroxide (NaOH) for 10 h and then in 30% hydrogen peroxide (H_2O_2) for 24 h, they were then washed with flowing tap water. The blocks were then immersed in the mixture of chloroform and methanol with 3:1 proportion for 1 h and dried at 70 °C for 24 h. The OCB was prepared well. Stage2. The pieces of OCB were sintered at 800 °C over a period of 6 h and maintained at that temperature for 3 h in a SiC-heated furnace to obtain porous ceramic-like ostrich cancellous bone (COB). Stage3. The pieces of COB were immersed in 0.1 mol/L sodium pyrophosphate (NP) for 24 h and dried at 70 °C for 48 h, then were sintered at 1100 °C over a period of 6 h and maintained at that temperature for 3 h in a SiC-heated furnace to obtain porous multiphasic calcium phosphate ceramic transformed by ostrich cancellous bone (OTBC) scaffolds. The pieces were cooled slowly to room temperature for next use. The crystallization phase of sample was analyzed by using X-ray diffractometer (XRD) (Rigaku D/Max-2400, Rigaku Corporation, Japan). The surface configuration of this material was observed by gross observation and scanning electron microscopy (SEM) (S-4800, Hitachi, Japan). The pore sizes and porosity of this material also be measured by the images of SEM [12].

2.3. Cell isolation and culture

Rabbit BMSCs were isolated and cultured as reported previously [13]. Briefly, BMSCs were isolated from the ilium marrow of adult rabbits. Iliac bone grafts were divided in half, and the marrow was flushed out with low-glucose Dulbecco's modified Eagle's medium (DMEM) (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (Gibco), 0.272 g/L L-glutamine (Sigma-Aldrich, St. Louis, MO, USA), and 2% antibiotics (200 mg/mL penicillin and 200 mg/mL streptomycin; Gibco). Clumps of cells were dispersed to achieve a homogeneous cell suspension by repeated pipetting. Then, the cell suspension was plated in 100-mm cell culture dishes, and standard medium was added to reach a volume of 15 mL. The cells were incubated at 37 °C in 5% CO_2 and 100% humidity. After 3 days, the medium and all floating cells were removed, and new medium was added to the remaining adherent cells, which were considered BMSCs. The medium was changed every 3 days until the cells reached confluence when the cells were subcultured at a ratio of 1:3.

Cell membranes were prepared by seeding MSCs of the second passage into 100-mm dishes at a density of 5 ×