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Fabrication and characterization of bioactive calcium silicate microspheres for drug delivery

Kaili Lin[a], Dong Zhai[a], Na Zhang[a], Naoki Kawazoe[b], Guoping Chen[b], Jiang Chang[a,*]

[a]State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, 1295 Dingxi Road, Shanghai 200050, China

[b]Tissue Regeneration Materials Unit, International Center for Materials Nanoarchitectonics, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan

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Abstract

The calcium silicate ($CaSiO_3$, CS) microspheres with diameter of 75–100 μm were fabricated by a spray-drying method. A new bone-like apatite layer fully covered the surface of the fabricated CS microspheres after soaking in simulated body fluid (SBF), suggesting the excellent activity of the material in inducing apatite deposition. The ionic extracts of CS microspheres promoted the proliferation of human osteoblast-like cells (MC3T3-E1). In addition, the porous structures of the CS microspheres resulted in favorable drug loading and sustained release property. Our study indicates that the fabricated multifunctional CS microspheres are a promising drug delivery system as an injectable bioactive filling material for bone-regeneration.
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Keywords: Calcium silicate; Microspheres; Bioactivity; Cell proliferation; Drug delivery

1. Introduction

Recently, the applications of bioactive microspheres as injectable bone filling implants and drug/cell carriers have received considerable attention [1–3]. After implantation, the spaces among the microspheres are crucial for effective and functional bone regeneration as they allow for both bone and vascular ingrowths [3]. Furthermore, compared with the traditional macroporous block scaffolds, the main advantage of the microspheres is that they not only possess better injectable and drug-delivery capacities but also the ability to fill bone defects with irregular and complex shapes and sizes [3,4].

However, as for the microspheres to be applied as bone fillers for bone regeneration, there are three major issues that need to be considered: (1) bioactivity, (2) sustainable drug release ability, and (3) degradability. Currently available microspheres are made of bioceramics, biopolymers, and their composites. Biopolymer-based microspheres such as polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA) and poly (hydroxybutyrate-polyhydroxyvalerate) (PHBV) are biodegradable but their bioactivity is unfavorable [5,6]. On the other hand, bioceramic microspheres such as hydroxyaptite bioceramics are bioactive, but lack adequate degradation [1].

Recently, Ca–Si-based silicate bioglasses and bioceramics have attracted considerable attention due to their excellent bioactivity and degradability [3,7–11]. They can quickly induce formation of a bone-like apatite layer on their surfaces after implantation *in vivo* [10]. This type of apatite layer plays an essential role in the formation of tight bone bonding between the bioactive materials and the host bone tissues [10–12]. The calcium silicate ($CaSiO_3$, CS) ceramics are biodegradable and possess excellent activity in inducing bone-like apatite layer formation *in vitro* and *in vivo* [10,13,14]. Our recent investigations demonstrated that the bioactive Si ions released from CS could provide a preferable extracellular environment for directing bone marrow mesenchymal stem cells (BMSCs) differentiation toward the osteogenic lineage, enhance human umbilical vein endothelial cells (HUVECs) proliferation and angiogenesis process even in the absence of extra osteogenic and angiogenic

*Corresponding author. Tel./fax: +86 21 52412804.
E-mail address: jchang@mail.sic.ac.cn (J. Chang).

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reagents, and significantly promote early bone formation compared with the traditional calcium phosphate bioceramics [15].

Many strategies have been developed to fabricate bioceramic microspheres, such as emulsion method [16], polymer mediated formation route [17], biomineralization technique [18], self-assembly approach [2,19], hard-template transformation process [20,21], and spray-drying method [22] among which the spray-drying method is particularly attractive because of its widespread use and relative ease of operation [23]. Moreover, the products with regulatable sizes from submicrometer to hundreds of micrometer-size, and polymorphic shapes, including mushroom-like and donut-like shapes, as well as spherical porous and hollow structures can be facilely controlled in a single step by modulation of the spraying parameters [22]. In addition, the products can be scaled up to ton quantities in one batch. In the present study, the bioactive CS microspheres as drug carriers have been developed using the spray-drying method for the potential application as injectable bone filling materials for bone regeneration. The antibiotic vancomycin model was selected in this study due to its broad spectrum of activity against both Gram-positive and Gram-negative bacteria that may induce bone infection. The fabrication method, morphology characterization, *in vitro* bioactivity, drug loading and delivery property, and the effect of the material extracts on cell proliferation are presented.

2. Experimental section

2.1. Fabrication and characterization of calcium silicate (CS) microspheres

The calcium silicate (CS) powders were prepared via a chemical precipitation method as previously described [7]. Briefly, 1000 mL of 0.5 mol $Na_2SiO_3 \cdot 9H_2O$ aqueous solution was added dropwise into 1000 mL of 0.5 mol $Ca(NO_3)_2 \cdot 4H_2O$ aqueous solution by vigorously stirring at room temperature to produce a white precipitate. After complete addition, the white precipitate was further stirred for 24 h followed by washing three times using distilled water, and then twice with 100% ethanol. After washing, the remaining liquid was removed by vacuum filtration, and the precipitate was dried at 120 °C for 24 h. The CS powders were obtained by calcining the dried precipitate at 900 °C for 2 h.

The CS microspheres were fabricated by the spray-drying method on a high-speed centrifugal spray-drying machine (LGZ-8, Wuxi Dongsheng, China). In brief, 100 g of CS powders was added into 500 g of aqueous solution containing 5 wt% polyvinyl alcohol (PVA) binders. Then the obtained CS suspension was atomized at a pressure of 1.5 MPa and a flow rate of 500 mL/h while the inlet and outlet temperatures of the nozzle were adjusted to around 180 °C and 80 °C, respectively. The spray-dried CS granules were collected and calcined at 900 °C for 3 h with a firing rate of 2 °C/min to burn out the PVA binders, and then cooled to room temperature in the furnace. Finally, the products were sieved to obtain the CS microspheres with 150–200 mesh. The hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HAp] microspheres were also fabricated by the spray-drying method using HAp nanopowders as raw materials. The HAp nanopowders were synthesized by the chemical precipitation method and then calcined at 800 °C for 2 h [24]. The obtained HAp microspheres were used as the control sample in the cell culture experiment.

The morphology and size of the fabricated CS microspheres were observed by scanning electron microscopy (SEM: JSM-6700F, JEOL, Japan). The phase of the microspheres was characterized by X-ray diffraction (XRD: D/max 2550 V, Rigaku, Japan) with mono-chromated Cu-Kα radiation.

2.2. Evaluation of bioactivity of CS microspheres in simulated body fluid (SBF)

The bioactivity of the fabricated CS microspheres was evaluated by examining the bone-like apatite layer formation on the surface of the microspheres in SBF [14], which was prepared as previously described by Kokubo and Takadama and had similar ion concentrations to those in human blood plasma [12]. Briefly, analytical reagent grade chemicals NaCl, $NaHCO_3$, KCl, K_2HPO_4, $MgCl_2$, $CaCl_2$, and Na_2SO_4 were dissolved in distilled water and the solution was buffered to pH 7.4 at 37 °C with tris(hydroxymethyl)aminomethane and hydrochloric acid (Tris–HCl).

The microspheres were soaked in SBF and vibrated at a constant speed of 240 rpm in a shaking air bath for 3 days at 37 °C with the ratio of mass (mg) to solution volume (mL) of 1.5. The SBF solutions were refreshed every 1 day. After soaking, the microspheres were removed from the SBF solution, gently rinsed with distilled water, and then dried at room temperature before further characterization. The formation of bone-like apatite layer on the surface of the microspheres was characterized by XRD, Fourier transform infrared spectroscopy (FTIR; A380 Nicolet Co., USA) and SEM.

2.3. Effect of ionic products from CS microspheres on MC3T3-E1 proliferation

The ionic extract method is a widely used international standard to evaluate the effect of chemical compositions on cell biological responses, which can effectively avoid the extra effects deriving from the material morphologies via direct incubation of materials with cells [2]. In this study, MC3T3-E1 mouse osteoblast cells were used, and the cells were cultured in Minimum Essential Medium α (MEM α, Invitrogen) containing 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin–streptomycin (PS; Gibco, USA). To prepare the extracts, a stock solution with a concentration of 200 mg/mL was first prepared by immersing the CS and HAp microspheres powders in MEM α culture medium. After incubation at 37 °C for 24 h, the mixtures were centrifuged and the supernatants were collected. The serial diluted extracts (100, 50, 25 and 12.5 mg/mL) were prepared by diluting the stock solutions with serum-free MEM α. Subsequently, these extracts were sterilized by filtration through 0.2 μm filter membranes for further cell culture experiments. The ion concentrations of the

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