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Hollow calcium silicate microspheres with a micro-porous structure for protein adsorption and local delivery

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ABSTRACT

Implants that simultaneously act as osteoconductive grafts and as devices for local drug delivery could provide an attractive system for bone regeneration and repair. In this work, we prepared hollow calcium silicate microspheres with open holes using a solid-in-oil-in-water (S/O/W) emulsion method, and the microspheres were modified with simulated body fluid (SBF) to introduce a micro-porous structure. We further studied the protein adsorption and release profile of two different model proteins, bovine serum albumin (BSA) and lysozyme (LSZ), for two kinds of microstructured microspheres. The SBF-modified microspheres adsorbed greater amounts of BSA and LSZ compared with pure calcium silicate microspheres, and the absorbed amount of BSA was greater than that of LSZ. The protein release profile was dependent on the initial protein loading and the microstructure of the microsphere. The results indicate that the hollow calcium silicate microspheres have significant potential as osteoconductive devices for local drug or growth factor delivery in bone regeneration.

1. Introduction

In the past few decades, there has been considerable interest in the development of implants for controlled local delivery of proteins and drugs [1]. Recently, microspheres have been proposed as a means of local drug delivery and have achieved markedly elevated pharmacological effects while minimizing systemic administration-associated toxic effects [2,3]. In the field of bone-related materials, an ideal system for bone regeneration should simultaneously function as a controlled local delivery device for drugs or growth factors and as an osteoconductive graft to support bone formation [4,5].

Calcium silicate is a synthetic biological ceramic used as an alternative autologous bone graft and drug-delivery microcarrier [6]. Calcium silicate has been reported to exhibit good biodegradability and osteoconductivity as a scaffold material for bone tissue engineering [7]. Until now, microspheres composed of bioceramics, such as hydroxyapatite [8,9] and bioglass [10,11], have been prepared for local drug delivery. However, the absence of open holes and the hollow structure of bioceramic microspheres have restricted their application for controlled drug release and bone regeneration [12,13]. Thus, it would be beneficial to fabricate open holes and hollow and micro-porous structures in calcium silicate microspheres for drug delivery and bone regeneration.

The objective of this study, therefore, was to develop hollow calcium silicate microspheres consisting of a hollow core and a micro-porous shell capable of carrying different kinds of protein. Bovine serum albumin (BSA) and lysozyme (LSZ) were selected based on their different molecular weights and electrostatic properties [14]. The ability to control the release kinetics of BSA and LSZ was studied by altering the microstructure of the shell of as-sintered calcium silicate microspheres using SBF modification.

2. Materials and methods

Materials: Calcium silicate, polyvinyl butyral (PVB), dichloromethane (DCM), poly(vinyl alcohol) (PVA), lysozyme (LSZ), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (MO, USA).

Preparation of hollow calcium silicate microspheres: Hollow calcium silicate microspheres were prepared from a calcium silicate powder by a solid-in-oil-in-water (S/W/O) emulsification technique. The powder (at 10 wt%) was added to an oil phase that consisted of DCM and PVB (5 wt%). The powder mixture solution was dropped into a water bath containing PVA at a concentration of 1 wt%, while stirring at 400 rpm, for 2 h. The dried microspheres were placed in an alumina crucible and heat treated in a furnace by subjecting the samples to the following conditions: heating to 600 °C for 2 h at a rate of 1 °C min^{-1}, followed by heating to 1100 °C for 2 h at a rate of

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5 °C min^{-1}; subsequently, the furnace was allowed to cool at room temperature. After sintering, the microspheres (500–700 μm) were sieved for the following experiments.

Characterization of the calcium silicate microspheres: The microstructure of the sintered ceramics was examined by scanning electron microscopy (SEM, JEOL, JSM-6460LV). The local composition of the external surface of the microspheres was determined using an energy dispersive X-ray (EDS) analysis in the SEM.

Surface mineralization: A sample with a weight of 25 mg was immersed in 1 mL of SBF [15] with an ion concentration nearly equal to that of human blood plasma for 1 day and 3 days to observe the growth of a bone-like apatite layer on the sample surface. After 1 day and 3 days, the SBF solution was removed, and the microsphere samples were rinsed with Milli-Q water. After drying overnight, these samples were coated with gold and examined by SEM.

BSA and LSZ loading: To determine the effect of the surface microstructure of the microspheres on protein adsorption and release, the microspheres treated with surface mineralization for 3 days (SBF-MS) and the as-sintered microspheres (MS) were compared.

Briefly, 100 mg of hollow calcium silicate microspheres was placed in a 1.5 EP tube, and 1 mL BSA or LSZ with a concentration of 1 mg mL^{-1} or 0.5 mg mL^{-1} in PBS, respectively was added. The level of protein loading was measured using a BCA protein assay (BCA™, Pierce, USA), with BSA as the standard, and a microplate reader (Multiscan Go 1510, Thermo Fisher Scientific). The protein capacities were expressed as the amount of adsorbed protein per gram of microsphere sample. All experiments were performed in triplicate.

Measurement of BSA and LSZ release profile in vitro: After the hollow calcium silicate microspheres were loaded with a solution of BSA or LSZ using the method outlined above, they were immersed in PBS to determine the release of BSA and LSZ from the microspheres. In the release step, 100 mg of microspheres was placed in a 1.5 EP tube containing 1 mL PBS. The system was kept at 37 °C and was subjected to continuous shaking. The concentration of BSA or LSZ in each aliquot was measured using the above-described BCA protein assay method.

3. Results and discussion

Characterization of the calcium silicate microspheres: Fig. 1a shows an optical image of the as-prepared calcium silicate microspheres prior to sintering. Spherical-shaped particles with open holes (red arrows) were successfully prepared. The hollow structure may have been created by the rapid evaporation of DCM solution during the formation of the microspheres. The rapid evaporation of DCM led to the solidification of the $CaSiO_3$/PVB composite microspheres. After sintering at 1100 °C for 2 h, the $CaSiO_3$ microspheres were prepared, and open holes with a size of

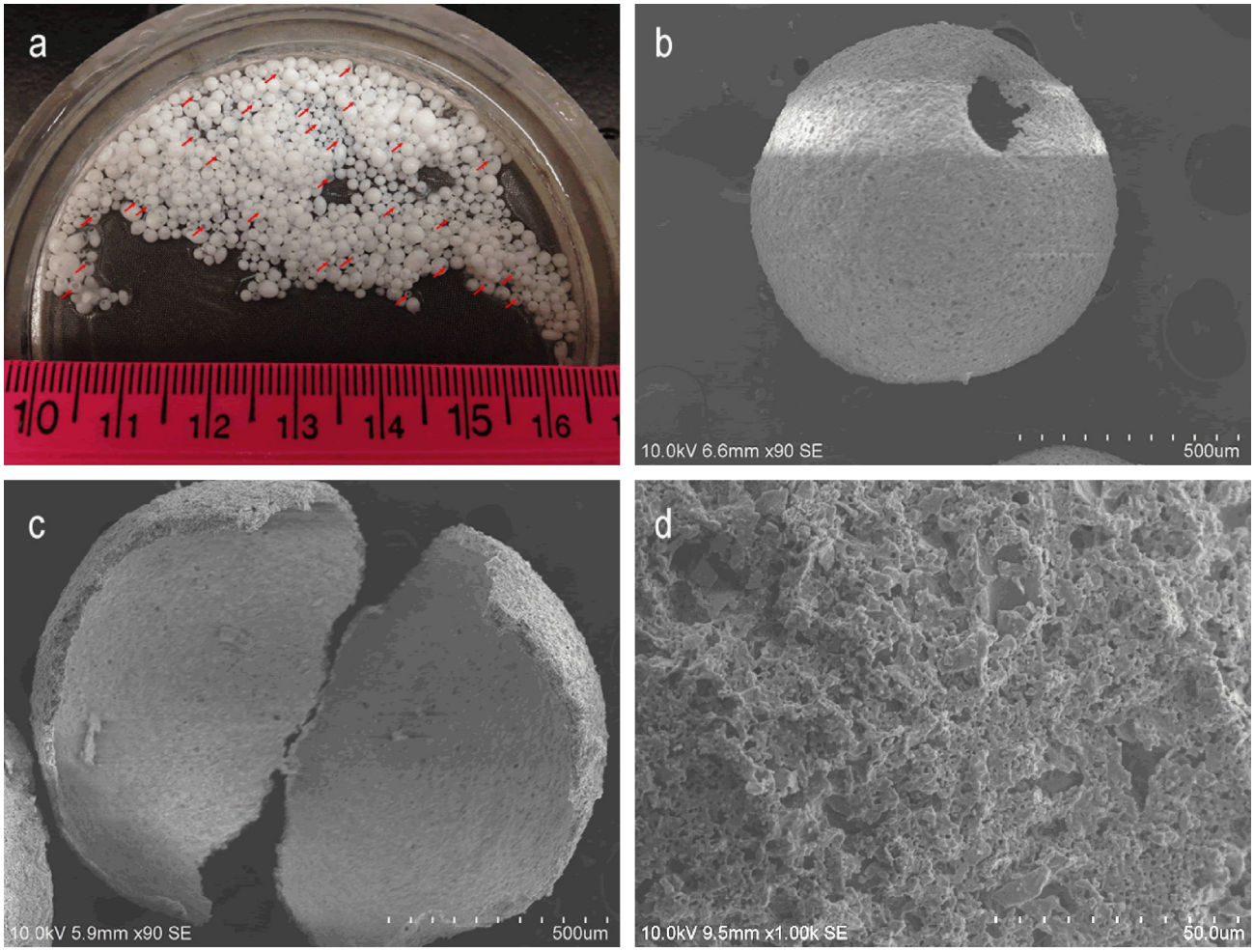

Fig. 1. Optical image of as-prepared calcium silicate microspheres before sintering (arrows indicate open holes on the shell) (a) and SEM images of hollow calcium silicate microspheres (b), cross section of the hollow structure (c), and external surface of the hollow microspheres at high magnification (d) after sintering. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article).