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Study of the mechanical property and in vitro biocompatibility of $CaSiO_3$ ceramics

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Abstract

Calcium silicate ($CaSiO_3$) ceramics were prepared by pressureless sintering. The effects of the sintering temperature and holding time on the mechanical strength, porosity and linear shrinkage of the ceramics, and the adhesion and proliferation of bone marrow mesenchymal stem cells (MMSC) were investigated. When the sintering temperature and holding time increased, the linear shrinkage of the ceramics increased and the porosity decreased. The $CaSiO_3$ ceramics sintered at 1100 °C for 5 h revealed a mechanical strength of 95.03 ± 7.11 MPa, similar to the bending strength of human cortical bone. In addition, the adhesion and proliferation of MMSC on the $CaSiO_3$ ceramics was examined and the results showed that the ceramics supported cell adhesion and proliferation, which indicated good biocompatibility. Our results suggested that $CaSiO_3$ ceramics might be a potential bioactive material as bone implant.

Keywords: A. Sintering; C. Mechanical properties; Calcium silicate; Ceramics; Biocompatibility

1. Introduction

It is known that some glasses and glass-ceramics such as A/W glass ceramics, Bioeutectic® and 45S5 Bioglass® are bioactive and can be used as bone implants in clinical applications [1–10]. One of the common characteristics of these materials is that they all contain $CaSiO_3$ or $CaO–SiO_2$ as a main bioactive component. Further studies have shown that pure $CaSiO_3$ powders and ceramics were highly bioactive and induced formation of a hydroxyapatite (HAp) layer on their surface after soaking in simulated body fluid (SBF) [11–14] or in human saliva [15]. This type of HAp layer plays an essential role in forming tight chemical bond between the bioactive materials and the bone tissue [6,16]. Siriphannon et al. have found that the rate of HAp formation on pure $CaSiO_3$ ceramic surface was faster than that on the biocompatible A/W glass ceramics and some other bioactive glass ceramics [12]. In addition, Liu et al. [17] and De Aza and co-workers [18,19] have reported that bioactive $CaSiO_3$ coatings could be prepared on titanium alloys. All these results suggest that $CaSiO_3$ ceramics may be a potential candidate for preparation of bioactive bone substitutes or bioactive coatings on metallic implants. However, all of the previous studies paid most of their attentions on the in vitro bioactivity in SBF, and less attentions on the sintering ability, the mechanical strength and the cellular biocompatibility of $CaSiO_3$ ceramics.

The objective of this work was to investigate the effect of the sintering processes, such as sintering temperature and holding time on the mechanical strength and microstructure of the $CaSiO_3$ ceramics. In addition, the in vitro biocompatibility of the $CaSiO_3$ ceramics was studied by examination of the adhesion and proliferation of the bone marrow mesenchymal stem cells.

2. Materials and methods

2.1. Preparation and sintering of the $CaSiO_3$ ceramics

$CaSiO_3$ powders for the present studies were synthesized by the reaction of $Ca(NO_3)_2{\cdot}4H_2O$ with $Na_2SiO_3{\cdot}9H_2O$.

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Briefly, 1000 ml of 0.4 mol $Ca(NO_3)_2{\cdot}4H_2O$ solution with a pH 11.4 was vigorously stirred at room temperature, and 1000 ml of 0.4 mol $Na_2SiO_3{\cdot}9H_2O$ was added dropwise over 40–60 min to produce a white precipitate. The white precipitate was then stirred for 12 h followed by washing four times with distilled water to remove the Na^+ and NO_3^- ions, and then washed two times with 100% ethanol to improve the dispersion characteristics. After washing, the remaining liquid was removed by vacuum filtration, and the precipitate was dried at 80 °C for 24 h, and than calcined at 800 °C for 2 h. The obtained $CaSiO_3$ powders were sieved to obtain 38–45 μm particulates, and mixed with 6 wt.% polyvinyl alcohol binders. The mixtures were uniaxially compacted at 14 MPa and subsequently cold isostatically pressed into rectangular-prism-shaped specimens (44 mm × 8 mm × 4 mm) under a pressure of 200 MPa for 15 min. Subsequently, they were sintered in air at selected temperatures in the range between 900 and 1250 °C from 1 to 15 h (heating rate 2 °C/min in all cases). The samples were cooled to room temperature in the furnace.

2.2. Characterization of the $CaSiO_3$ ceramics

The fracture surfaces of the sintered ceramics were observed by a field emission scanning electron microscope (FESEM; JSE-6700F, JEOL, Japan). The porosity of the sintered materials was measured by the Archimedian method. The three-point bending strength of the sintered materials was measured at the mechanical testing machine with a loading rate of 0.5 mm/min according to the JIS R1601 standard (Shimadza AG-5kN, Japan). A span length of 30 mm and rectangular-prism-shaped specimens (36 mm × 6 mm × 3 mm) with surfaces polished by 0.5 μm diamond powders were used. In the study, five pieces of samples were used to test the average mechanical strength, porosity and linear shrinkage in each group.

2.3. Culture of the bone marrow mesenchymal stem cells on the sintered $CaSiO_3$ ceramics

Primary bone marrow mesenchymal stem cells (MMSC) from newborn calf femur (less than 1 day old) were isolated and cultured using the method described by Maniatopoulos [20]. The cells were cultured in Dulbecco's Modified Eagle Medium (Invitrogen) supplemented with 10% FCS (fetal calf serum, Gibco), 100 U/mL penicillin G and 100 μg/mL streptomycin (Invitrogen). The culture medium was regularly changed twice a week. For this investigation only cells at the 4–5th passage were employed. The $CaSiO_3$ ceramic disks were sterilized under ultraviolet light. After washing with sterile phosphate-buffered saline (PBS pH 7.4) and prewetting with culture media, ceramic discs were seeded with the MMSC at a density of 2000 cells/cm^2, and the culture was maintained at 37 °C in humidified atmosphere of 95% air and 5% CO_2 for 1, 3 and 5 days, respectively. At the selected time points, disks were removed from the culture wells. For morphological examination, samples were rinsed with PBS twice and fixed with 2.5% glutaraldehyde solution in a sodium cacodylate buffer (pH 7.40) for 30 min. After fixation, the samples were rinsed with PBS once and then dehydrated in a grade ethanol series (70, 90, 96 and 100%) for 10 min, respectively. Subsequently, the samples were dried in hexamethyldisilizane (HMDS, Shanghai Chemical Co., China) ethanol solution series [21], sputter-coated with gold and then viewed by FESEM. The other disks were rinsed with PBS twice to eliminate unattached cells, fixed with 100% methanol for 10 min and then stained in fresh Giemsa for 15 min. After staining, the samples were rinsed with distilled water and dried in air. The adherent cells (on each of six random fields per substrate) were visualized and counted under a light microscope (Leica S6D, Germany). The average cell count per substrate was expressed as cells/cm^2 of substrate surface area. Data were analysed for statistical significance using an analysis of variance. Differences at P values of less than 0.05 were considered significant.

3. Results and discussion

The FESEM micrographs of the fractured surface of the samples sintered at 1100 °C for 3 h, 15 h and at 1250 °C for 3 h are shown in Fig. 1A–C, respectively. The grain size of the samples sintered at 1100 °C for 3 h were about 1–2 μm (Fig. 1A). When the sintering temperature increased, the grain growth was observed, and the grain size of the samples sintered at 1250 °C was >10 μm (Fig. 1B). On the other hand, the increase of the holding time from 3 to 15 h also resulted in the grain growth of the samples (Fig. 1C). Most of the grain size increased from 1–2 to 5–10 μm, which

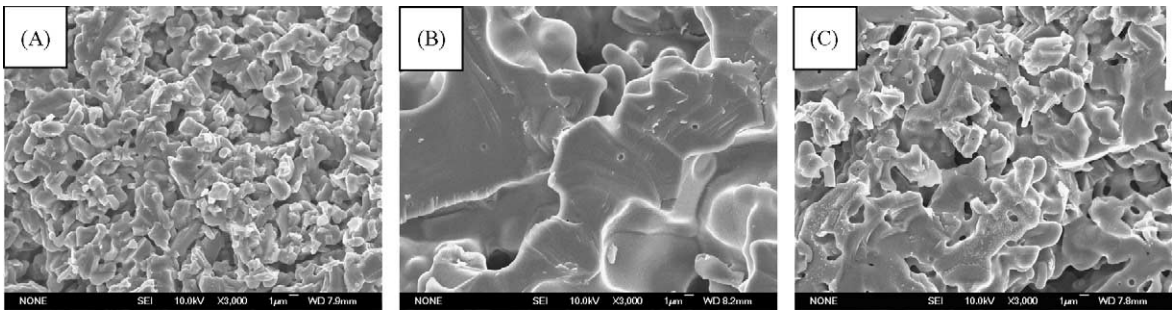

Fig. 1. The micrograph of samples sintered at 1100 °C for 3 h (A), 1250 °C for 3 h (B) and 1100 °C for 15 h (C).