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A novel hardystonite bioceramic: preparation and characteristics

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Abstract

Hardystonite ($Ca_2ZnSi_2O_7$) ceramics were prepared by sintering hardystonite sol–gel derived powder compacts at 1350 °C for 5 h, and their sinterability and mechanical properties were investigated. The biocompatibility of hardystonite ceramics was evaluated by culturing bone marrow mesenchymal stem cells (BMMSC) on the ceramics. The results showed the bending strength and fracture toughness of hardystonite ceramics to be 136 MPa, and 1.24 MPa $m^{1/2}$, respectively. SEM showed that BMMSC were attached and spread well on the hardystonite ceramics. Our preliminary studies indicate that hardystonite ceramics possess improved bending strength and fracture toughness as compared to hydroxyapatite (HAp), and may possess good biocompatibility.

Keywords: A. Sol–gel processes; Hardystonite; Biocompatibility

1. Introduction

Previous studies showed that some Ca, Si containing bioactive glass, glass-ceramics and ceramics were biocompatible [1–3] and could induce hydroxyapatite (HAp) formation in body fluid environment [4]. Zn was reported to be involved in bone metabolism [5–11]. Zn could stimulate bone formation and increase bone protein, calcium content, and alkaline phosphatase activity in humans and animals [5–10]. Ito et al. [12] found that Zn in biphasic Zn tricalcium phosphate (Zn-TCP)-apatite ceramics could enhance cell proliferation. In addition, Zn containing Ca–P ceramics could enhance the osteoconductivity and had a pharmaceutical effect on promoting bone formation [13,14]. Recently, Ikeuchi et al. [15] reported that ZnTCP/HAp ceramics promoted osteogenic differentiation of bone marrow cells. Furthermore, a hybrid Zn–Ca–Si polyalkenoate bone cement was prepared and appeared to be a potential candidate for orthopedic restoration [16]. Hardystonite ($Ca_2ZnSi_2O_7$) is a mineral containing Ca, Zn, and Si, with a melting temperature of 1425 °C and a density of 3.40 g/cm^3, and so far has no important industrial applications. Considering chemical composition, hardystonite might be biocompatible and used as biomaterials. To our knowledge, there was no report about preparation of hardystonite ceramics.

In this study, hardystonite powders were synthesized by the sol–gel method, and hardystonite ceramics were prepared by uniaxial pressing of the hardystonite powders and sintering at different temperatures. In addition, the mechanical properties and sinterability of hardystonite ceramics were also evaluated. At last, the biocompatibility of hardystonite ceramics was investigated.

2. Experimental procedure

2.1. Synthesis of hardystonite powders

Hardystonite powders were prepared by the sol–gel process using tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS), zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$) and calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) as raw materials. Briefly, the TEOS was mixed with water and 2 M HNO_3 (mol ratio: $TEOS/H_2O/HNO_3 = 1{:}8{:}0.16$) and hydrolyzed for 30 min under stirring. Then, the $Zn(NO_3)_2{\cdot}6H_2O$ and $Ca(NO_3)_2{\cdot}4H_2O$ were added into the mixture (mol ratio: $TEOS/Zn(NO_3)_2{\cdot}6H_2O/Ca(NO_3)_2{\cdot}4H_2O = 2{:}1{:}2$), and reactants were stirred for 5 h at room temperature. After the

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reaction, the solution was maintained at 60 °C for 1 day and dried at 120 °C for 2 days to obtain the dry gel. The dry gel was ground and sieved to 250-mesh, transferred into a corundum crucible and calcined at 1100 and 1200 °C for 3 h, respectively.

The calcined powders were analyzed by X-ray diffraction (XRD, Geigerflex, Rigaku Co., Japan) with a monochromated Cu Kα radiation, and the microstructure of calcined powders was observed by scanning electron microscopy (SEM; JSM-6700F, JEOL, Tokyo, Japan).

2.2. Preparation of hardystonite ceramics

Hardystonite ceramics were prepared by uniaxial pressing of the hardystonite powders at 10 MPa followed by isostatic pressing at 200 MPa and sintered at different temperatures. To evaluate mechanical properties and sinterability, green bodies of hardystonite of 45.5 mm × 8.0 mm × 3.5 mm in size were prepared and fired from 1250 to 1350 °C. The sintered hardystonite ceramics were analyzed by XRD and SEM.

For evaluation of in vitro biocompatibility, ceramic discs of 6 mm Ø × 1.5 mm in size were uniaxially pressed at 10 MPa followed by isostatic pressing at 200 MPa, and sintering at 1350 °C for 5 h.

2.3. Characterization of the hardystonite ceramics

2.3.1. Mechanical properties and sinterability of hardystonite ceramics

Ceramic bars sintered at different conditions were polished with diamond paste. Bending strength was applied normally with a deflectometer (AG-5KNL, Shimadzu Co., Japan) at a crosshead speed of 0.5 mm/min. To measure the fracture toughness, the samples were ground and notched 3.00 mm deep with a 0.1-mm-thick diamond wheel, and the fracture toughness was evaluated by a material testing machine (Instron 5566, USA) through single notched precrack bending (SNPB) method.

The length of samples before and after sintering was measured, and the linear shrinkage of hardystonite ceramics was calculated. The apparent density of hardystonite ceramics was measured in water using the Archimedes' technique.

2.3.2. BMMSC attachment on hardystonite ceramics

Bone marrow mesenchymal stem cells (BMMSC) were used to evaluate the biocompatibility of the hardystonite ceramics. BMMSC were isolated from bone marrow of healthy newborn calf femur, as described by Maniatopoulos et al. [17]. After the bone was sawed open, BMMSC were collected and cultured in a flask containing 10 ml Dulbecco's modified Eagle's medium-low glucose (Gibco) with 10% fetal calf serum plus antibiotics at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. For this investigation only the cells at the 3th–5th passage were employed.

The cells were seeded on each disc at a density of 2.5×10^4 cells/cm^2 in a 48-well plate and incubated for 3 days in DMEM culture medium supplemented with 10% calf serum maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. After different time of culture, the discs were removed from the culture wells and rinsed with phosphate-buffered saline (pH 7.2, PBS) twice to remove unattached cells and fixed with 2.5% glutaraldehyde solution in a sodium cacodylate buffer (pH 7.40) for 30 min. For SEM observation, the discs were dehydrated in a grade ethanol series (70, 90, and 96% v/v) for 10 min, respectively, with final dehydration in absolute ethanol twice and followed by drying in hexamethyldisilizane (HMDS) ethanol solution series [18].

2.4. Statistical analysis

Triplicate experiments were performed. The results are shown as the arithmetic mean ± standard deviation (±S.D.). Analysis of the results was carried out using *t*-test, with a significance level of $P < 0.05$.

3. Results and discussion

3.1. Characterization of hardystonite powders

Hardystonite is a mineral, and the naturally occurring hardystonite was often not pure and associated with other minerals. To our knowledge, there was no report about chemical synthesis of pure hardystonite powders. Here we report a study on chemical synthesis of pure hardystonite powders by sol–gel method. Fig. 1 shows the XRD patterns of the hardystonite powders prepared by this method. A strong hardystonite peak at about 31° 2θ was obvious in the XRD patterns of the powders calcined at 1100 and 1200 °C, which indicated that the main resultant was hardystonite. However, at the temperature of 1100 °C, the calcined powders contained impurities of willemite (Zn_2SiO_4) (Fig. 1a). When the

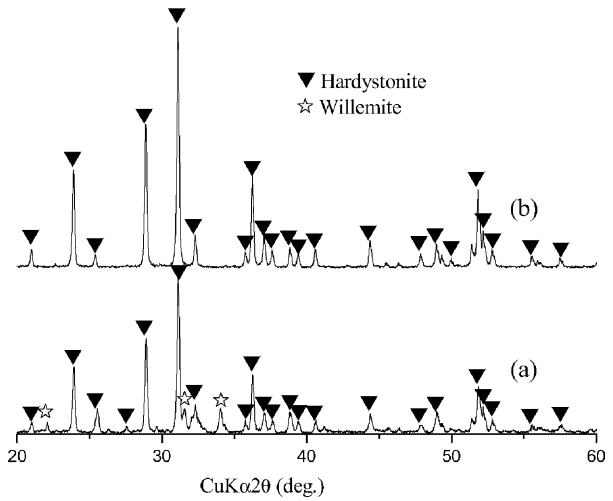

Fig. 1. XRD patterns of the hardystonite powders prepared by the sol–gel method and calcined at: (a) 1100 °C and (b) 1200 °C.

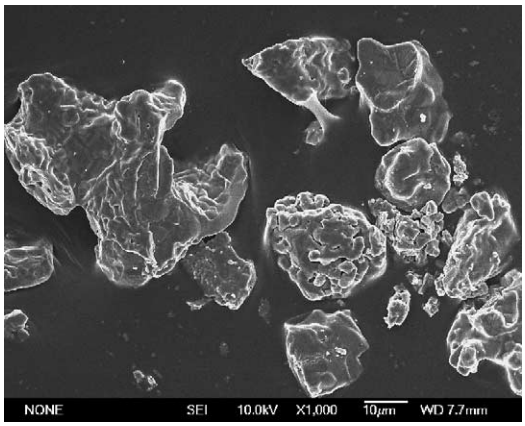

Fig. 2. SEM photographs of microstructures of the hardystonite powders calcined at 1200 °C.

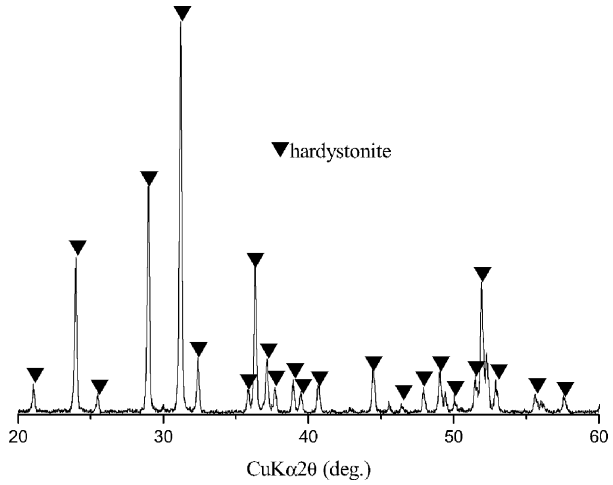

Fig. 3. XRD pattern of hardystonite ceramics sintered at 1350 °C for 5 h.

temperature increased to 1200 °C, pure hardystonite powders were obtained (Fig. 1b). The morphology of the prepared powders is shown in Fig. 2, which indicated that the particles agglomerated and the size of particles was about 5–40 μm. Our results indicated that pure hardystonite powders were synthesized by sol–gel method and calcining at 1200 °C, and the calcination temperature was critical to the purity of the prepared powders.

3.2. Phase composition, microstructure and sinterability of hardystonite ceramics

Fig. 3 shows the XRD pattern of the ceramics showing only the pattern of hardystonite. The surface morphology of the sintered hardystonite ceramics is shown in Fig. 4.

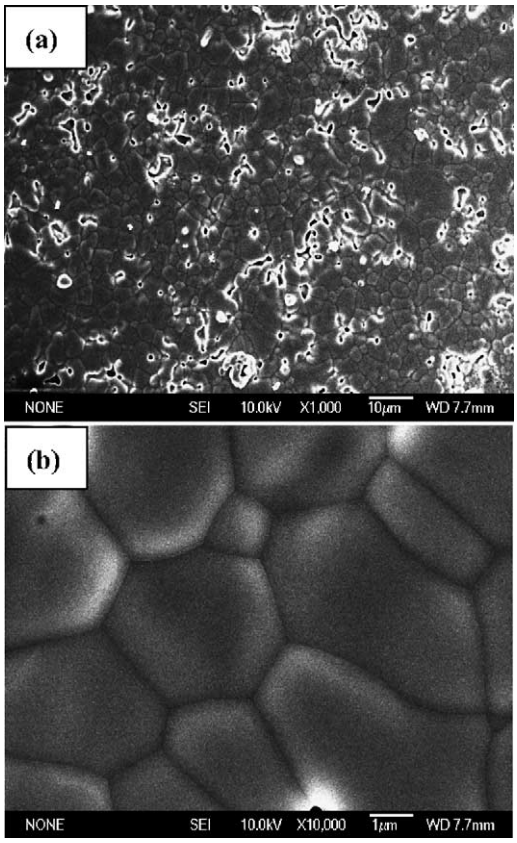

Fig. 4. Surface morphology of the sintered hardystonite ceramics at 1350 °C for 5 h.

It can be seen that most hardystonite particles were sintered and some micropores were evident (Fig. 4a), which indicated that the sintered hardystonite ceramics were not completely dense. The high magnification SEM micrograph shows that the grain size of hardystonite ceramics was about 1–5 μm (Fig. 4b). In this study, the relative density and linear shrinkage of hardystonite ceramics were studied, and results in function of the sintering temperatures are shown in Table 1. An increase of the sintering temperature from 1250 to 1350 °C resulted in an increase of the relative density from 63.5 to 76.5% ($P < 0.05$) and of linear shrinkage from 4.0 to 5.9% ($P < 0.05$). On the other hand, as shown in Table 2, an increase of the sintering time from 1 to 5 h resulted in an increase of the relative density from 72.9 to 82.6% ($P < 0.05$) and of linear shrinkage from 4.8 to 7.7% ($P < 0.05$), which indicated that the sintering time was also an important factor to affect densification. From the results above, we can see that the optimal sinter-

Table 1
Changes of the relative density, linear shrinkage, bending strength, and fracture toughness of the fired bodies sintered at different temperatures for 3 h

Sintering temperature (°C)	Relative density (%)	Shrinkage (%)	Bending strength (MPa)	Fracture toughness (MPa m$^{1/2}$)
1250	63.5 ± 1.1	4.0	54.1 ± 2.9	0.76 ± 0.01
1300	67.0 ± 2.4	4.2	69.6 ± 2.3	0.77 ± 0.01
1350	76.5 ± 1.4	5.9	102.2 ± 8.1	0.98 ± 0.05

Table 2
Changes of the relative density, linear shrinkage, bending strength, and fracture toughness of the fired bodies sintered at different time at 1350 °C

Sintering time (h)	Relative density (%)	Shrinkage (%)	Bending strength (MPa)	Fracture toughness (MPa $m^{1/2}$)
1	72.9 ± 1.5	4.8	74.3 ± 2.7	0.85 ± 0.02
3	76.5 ± 1.4	5.9	102.2 ± 8.1	0.98 ± 0.05
5	82.6 ± 2.3	7.7	136.4 ± 3.9	1.24 ± 0.03

ing condition to densification of hardystonite ceramics was 1350 °C for 5 h.

3.3. Mechanical properties of hardystonite ceramics

The changes of mechanical properties of hardystonite ceramics with relative density are shown in Tables 1 and 2. The increase of density from 63.5 to 76.5% resulted in an increase of the bending strength from 54.1 to 102.2 MPa ($P < 0.05$) whereas the same effect could not be observed on fracture toughness, which varied only from 0.76 to 0.98 MPa $m^{1/2}$ ($P < 0.05$) (Table 1). When the relative density increased from 72.9 to 82.6%, the bending strength increased from 74.3 to 136.4 MPa ($P < 0.05$), and fracture toughness also significantly increased from 0.85 to 1.24 MPa $m^{1/2}$ ($P < 0.05$) (Table 2). From the results above, we can see that the optimal mechanical properties of hardystonite ceramics were 136.4 MPa for bending strength and 1.24 MPa $m^{1/2}$ for fracture toughness. The bending strength of HAp has been reported to vary between 80 and 89.07 MPa and the fracture toughness between 0.75 and 1.0 MPa $m^{1/2}$ [19–21]. Our study showed that the hardystonite ceramics possessed improved mechanical properties compared to HAp.

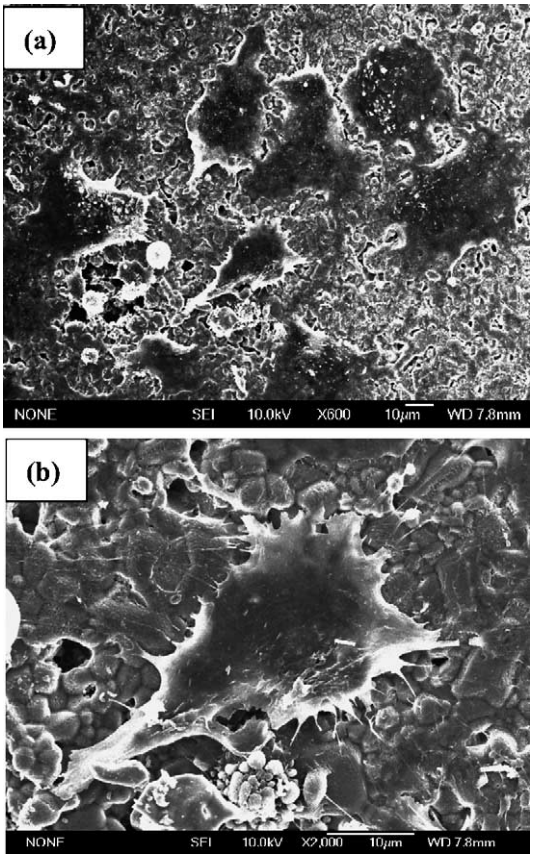

Fig. 5. SEM morphology of hardystonite ceramics seeded with BMMSC and cultured for 3 days.

3.4. Biocompatibility of hardystonite ceramics

The morphology of hardystonite ceramics seeded with BMMSC and cultured for 3 days is shown in Fig. 5. BMMSC attached and spread well on the surface of disc after 3-day culture (Fig. 5a). The high magnification SEM micrograph shows the cell to exhibit flattened morphology, and minor filopodia could be observed (Fig. 5b). The attachment and spreading of the cells on biomaterials are important processes of the cell/material interactions [22,23]. Our results showed that BMMSC have completely attached and spread on the hardystonite ceramics. Preliminary studies indicate that hardystonite ceramics may possess good biocompatibility.

4. Conclusions

Pure hardystonite ($Ca_2ZnSi_2O_7$) powders were synthesized by the sol–gel method and hardystonite ceramics were prepared by sintering powder compacts. Best results were obtained at 1350 °C for 5 h, where the bending strength was 136 MPa and the fracture toughness 1.24 MPa $m^{1/2}$. In the in vitro study, BMMSC attached and spread on the hardystonite ceramics. Our preliminary studies indicate that hardystonite ceramics possess improved bending strength and fracture toughness as compared to HAp, and may possess good biocompatibility.

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