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Preparation and characterization of a novel willemite bioceramic

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Abstract Willemite (Zn_2SiO_4) ceramics were prepared by sintering the willemite green compacts. The effects of sintering temperature on the linear shrinkage, porosity and mechanical strength of the ceramics were examined. With the sintering temperature increased, the linear shrinkage of the ceramics increased and the porosity decreased. When sintered at 1,300°C, willemite ceramics showed mechanical properties of the same order of magnitude as values for human cortical bone, as measured by bending strength (91.2 ± 4.2 MPa) and Young's modulus (37.5 ± 1.5 GPa). In addition, the adhesion and proliferation of rabbit bone marrow stromal cells (BMSCs) on willemite ceramics was investigated. The results showed that the ceramics supported cell adhesion and stimulated the proliferation. All these findings suggest that willemite ceramics possess suitable mechanical properties and favorable biocompatibility and might be a promising biomaterial for bone implant applications.

1 Introduction

Since Hench et al. [1] first synthesized 45S5 Bioglass®, Silicon(Si)-containing biomaterials have attracted growing attention. Succedent investigations further confirmed that the ionic products of these Si-containing biomaterials such as bioglass and silicate bioceramics promoted osteoblastic gene and protein expression [2–4]. Earlier studies have suggested that silicon was an essential element in skeletal development. Calrisle [5] first reported in the 1970s that silicon was uniquely localized in the active areas of young bone and involved in the early stage of bone calcification. He also found that silicon enhanced chicks growth, and silicon-deficient chicks showed retarded skeletal development and skull deformations [6]. These effects were also observed by Schwarz and Milne [7] in silicon-deficient rats.

Zinc(Zn) is also one of the essential trace elements in humans and other animals [8, 9]. It is closely associated with the growth, development, and maintenance of healthy bones. Bone growth retardation and defects are common findings in humans and animals with zinc deficiency [10–12]. Yamaguchi [13] has reported that zinc has a stimulatory effect on bone formation and mineralization and moreover, zinc inhibits osteoclastic bone resorption. Recently, zinc-doped calcium phosphate [14–16] and ZnO-containing bioactive glasses [17–19] have been studied. The results showed that the Zn-containing biomaterials with certain Zn concentration could stimulate the osteoblastic activity and facilitate new bone formation.

It has been proved that silicate bioceramics are promising candidates for bone replacement and regeneration applications [20–22]. Hardystonite ($Ca_2ZnSi_2O_7$), a zinc-containing silicate, has been prepared and showed favored human osteoblast-like cells attachment, cytoskeleton organization, proliferation and differentiation [23, 24]. Therefore, it is worthy to develop new-type of zinc-containing silicate bioceramics so as to meet diversified clinical needs.

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Willemite (Zn_2SiO_4), an important ceramic in the zinc-silica system, has been widely studied as luminescent and dielectric material [25–27]. However, there is no report about its biological function and application. The objective of this study is to fabricate willemite ceramics, investigate their sinterability and mechanical properties, and especially their in vitro biocompatibility.

2 Materials and methods

2.1 Preparation of willemite ceramics

Willemite powders were synthesized by the solid reaction process using ZnO (99.99%) and SiO_2 (99.9%) as raw materials according to the procedure described by Guo [27]. Briefly, ZnO powders were mixed with SiO_2 powders by ball milling in ethanol for 24 h. After drying at 60°C, the mixed powders were ground and then calcined at 1,250°C for 2 h.

The synthesized powders were uniaxially pressed at 10 MPa to form a rectangular compact (45.5 mm × 8.0 mm × 3.5 mm) using 6% polyvinyl alcohol (PVA-124) as a binder, then followed by a cold isostatic pressing at 200 MPa. The green samples were subsequently sintered at 1250, 1300 and 1350°C for 2 h, respectively, with a heating rate of 2°C/min.

For cell culture experiments, ceramic discs with the dimension of Φ8 mm × 2 mm were prepared under 10 MPa by uniaxial pressing, and sintered at 1,300°C for 2 h.

2.2 Characterization of willemite ceramics

The sintered samples were analyzed by X-ray diffraction (XRD, Geigerflex, Rigaku, Japan) with a monochromated CuKα radiation, and the microstructure of sintered samples was observed by scanning electron microscopy (SEM; JSM-6700F, JEOL, Japan). The linear shrinkage was calculated from the length of the samples before and after sintering. The porosity was measured by the Archimedes principle according to ASTM C-20. The 3-point bending strength and Young's modulus were measured with a mechanical testing machine (AG-5kNL, Shimadzu Co, Japan) at a crosshead speed of 0.5 mm/min with a span length of 30 mm according to the JIS R1601 standard. In this study, five samples of each group were used to test the average linear shrinkage, porosity and mechanical strength.

2.3 Cell culture

Bone marrow stromal cells (BMSCs) were isolated from tibias of adult New Zealand white rabbits. After the bone was excised under sterile condition, fresh bone marrow was collected aseptically and suspended in cell culture dishes containing 10 ml Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) with 10% (v/v) heat-inactivated fetal calf serum (FCS) plus antibiotics. After 10 days of culture at 37°C in a humidified atmosphere of 95% air and 5% CO_2, hematopoietic and other floating cells were removed from the dishes by repeated washing with phosphate-buffered saline (PBS, pH = 7.4). Culture media were refreshed every 2 days until the primary BMSCs reached confluency. The cells were routinely subcultured by trypsinization. For this study, the cells between the 3rd and 5th passage were employed.

2.4 Cell adhesion assay

Willemite ceramic disks were sterilized by autoclave. The cells were seeded onto the disks at a density of 2×10^4 cells/disk in a 48-well tissue culture plate (TC plate) and incubated in DMEM supplemented with 10% FCS maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2. After incubation for 24 h, the disks were rinsed with PBS twice, placed in 10% formalin solution for 30 min and stained with crystal violet (0.04%) for light microscopy observation. For SEM observation, the disks after fixation were dehydrated in a grade ethanol series ((v/v); 30, 50, 70, 90, 95 and 100% for 10 min each). The samples were treated in a 50% alcohol-HMDS (hexamethyldisilazane; Shanghai Chemical Reagent Co.) solution (v/v) for 10 min, then in pure HMDS for 10 min, and finally air dried in a desiccator overnight [28]. The specimens were then sputter-coated with gold and viewed by SEM.

2.5 Cell proliferation assay

For the examination of cell proliferation, cells were seeded onto the ceramic discs at a density of 2×10^4 cells/well in a 48-well plate. The TC plate without ceramic disks was as blank control. Culture medium was replaced every other day. Cell proliferation was tested after incubation for 1 day and 7 days by MTT method. In brief, 1 ml of 0.5 mg/ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma, USA) solution was added in each well. After additional incubation for 4 h, di-methyl sulfoxide (DMSO) was added to stop the reaction between MTT and cells. The optical density (OD) was measured at the wavelength of 490 nm using an enzyme-linked immunoadsorbent assay plate reader (ELx 800, BIO-Tek, USA). Five specimens for each group were tested. Meanwhile, the ion concentrations of medium with willemite ceramic after 7 days of culture were measured by inductively plasma atomic emission spectroscopy (ICP-AES; Varian Co., USA).

2.6 Statistical analysis

Results were expressed as the arithmetic means ± standard deviation (SD) for $n = 5$ and were analyzed using Student's t test. A P value < 0.05 was considered statistically significant.

3 Results and discussion

3.1 Characterization of willemite ceramics

Figure 1 shows the XRD patterns of willemite ceramics sintered at the temperature of 1,250 and 1,350°C. Only sharp peaks of willemite (JCPDS card: No. 83-2270) are observed, which indicated the samples are phase-pure and well crystallized. This result is in accordance with that Guo [27] reported.

Table 1 lists the linear shrinkage, relative density and mechanical properties of willemite ceramics sintered at different temperature. With the increase of the sintering temperature, the linear shrinkage of the ceramics increased and the porosity decreased. The optimal bending strength (91.2 ± 4.2 MPa) and Young's modulus (37.5 ± 1.5 GPa) of the ceramics were obtained at 1,300°C, which were analogous to the values of human cortical bone [29]. With a further increase of the sintering temperature up to 1,350°C, the mechanical strength decreased. In general, the porosity and grain size are two key factors affecting the strength of the ceramics. The strength of ceramics decreases with an increase in porosity and grain size [30, 31]. When the sintering temperature increased from 1,250 to 1,300°C, the porosity of the samples decreased obviously as shown in Fig. 2a, b, which was the main reason causing the significant improvement of the strength of the samples. However, when the temperature rose to 1,350°C, no obvious change of porosity happened comparing Fig. 2b, c, while the grain growth was obvious, which led to the decrease of mechanical strength. Therefore, the optimum sintering temperature is 1300°C for the best mechanical strength of willemite ceramics.

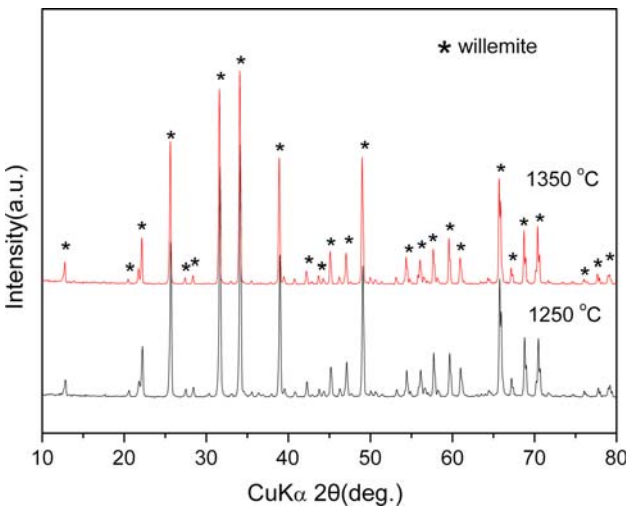

Fig. 1 XRD patterns of sintered willemite ceramics

Table 1 Linear shrinkage, relative density and mechanical properties of willemite ceramics sintered at different temperature for 2 h

Sintering temperature (°C)	Line shrinkage (%)	Relative density (%)	Bending strength (MPa)	Young's modulus (GPa)
1,250	6.9 ± 0.2	82.6 ± 0.8	53.2 ± 3.3	25.4 ± 2.9
1,300	11.2 ± 0.0	96.7 ± 0.2	91.2 ± 4.5	37.5 ± 1.5
1,350	11.9 ± 0.2	97.7 ± 0.2	65.9 ± 4.6	30.9 ± 3.4

3.2 Adhesion of BMSCs on willemite ceramics

Light micrograph (Fig. 3a) showed the morphological features of BMSCs that adhered and spread on willemite ceramics for 24 h. It could be seen that cells adhered to the sample surface and spread with elongated and flatted shapes. In addition, from the high magnification SEM micrograph (Fig. 3b) cells were observed to spread well with an intimate contact with the surface of the ceramics, and it was clear to see that the cells with their thin cytoplasmic digitations adopted typical trigonal morphology on the materials. This result suggests that willemite ceramics have good biocompatibility to support cell adhesion.

3.3 Proliferation of BMSCs on the ceramics

MTT test showed that cells on willemite ceramics proliferated obviously with the increase of culture time (Fig. 4). Furthermore, cells on ceramics presented a higher proliferation rate ($P < 0.05$) compared with the blank group after 7 days of culture. The ion concentrations of Si and Zn in culture medium on the 7th day were shown in Table 2. Previous studies have shown that zinc could promote bone formation and the stimulatory effect was observed in a range of zinc concentrations from 10^{-6} to 10^{-3} M [32, 33]. In our work, zinc released from willemite ceramic measured on the 7th culture day was 1.2×10^{-5} M, which is exactly between 10^{-6} and 10^{-3} M. Therefore, it is believed that zinc ions released from ceramics play an important role for the stimulation of cell proliferation. The ion concentration of Si released from willemite was 0.015 mM, which was within physiological concentrations (0.005–0.02 mM) of Si in plasma as reported by Reffitt et al. [34] and he showed that orthosilicic acid at physiological concentrations stimulated collagen typeIsynthesis in human osteoblast-like cells and enhanced osteoblastic differentiation.

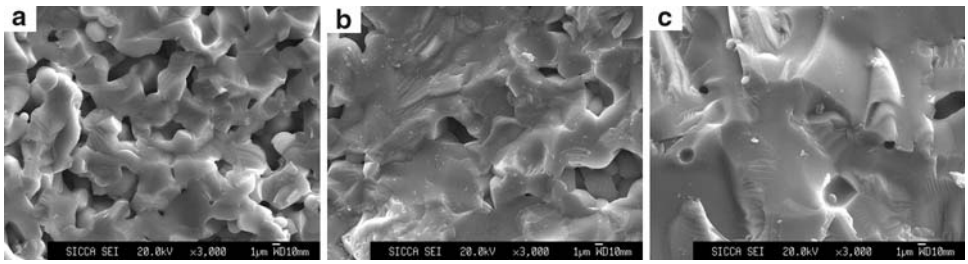

Fig. 2 SEM micrographs of the fractured surface of samples sintered at 1250°C (**a**), 1300°C (**b**) and 1350°C (**c**)

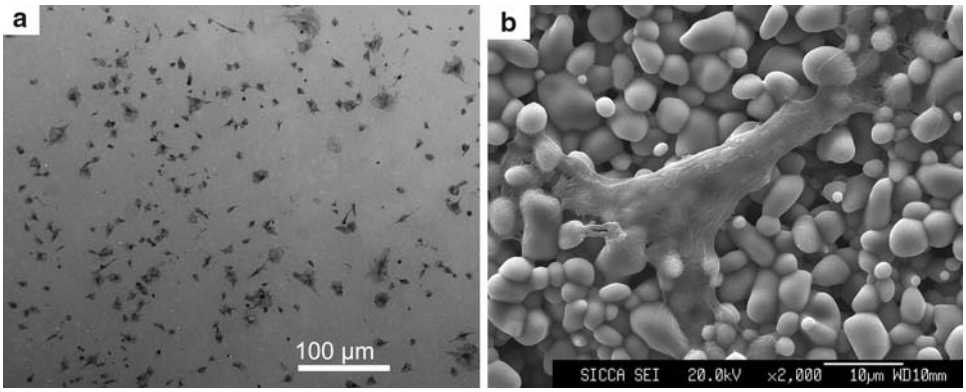

Fig. 3 Morphology observation of BMSCs on willemite disks after seeding for 24 h. **a** light micrograph; **b** SEM micrograph

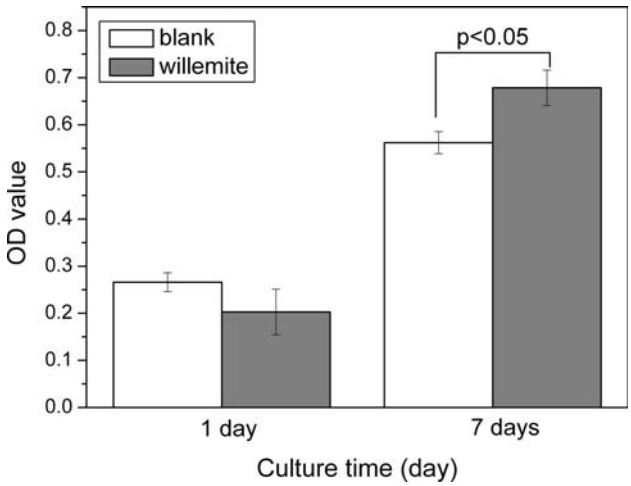

Fig. 4 The cell proliferation on willemite ceramics and tissue culture plate (blank) by MTT assay for 1 day and 7 days

Table 2 Ion concentrations of culture medium after 7 days of culture (mM)

Samples	Zn	Si
Blank	0	0
Willemite	0.012	0.015

Moreover, the results of many studies also suggested that Si-containing ionic products of biomaterials such as bioactive glass [3, 35] and silicate bioceramics [4, 36] could stimulate osteoblastic proliferation. As a Si- and Zn-containing binary ceramic, our results suggest that willemite ceramics have good biocompatibility and the ionic products of the ceramic could promote cell proliferation.

4 Conclusions

In this study, willemite ceramics were prepared by sintering willemite powder compacts. The optimal bending strength (~91 MPa) and Young's modulus (~37 GPa) of willemite ceramics were similar to those of human cortical bone. The in vitro study showed that the ceramic supported bone marrow stromal cells adhesion and spreading. Moreover, MTT tests demonstrated that cells on willemite ceramics exhibited a significantly higher proliferation rate than on the controls after 7 days of culture. In conclusion, willemite ceramics possessed favorable mechanical properties and excellent biocompatibility and might be suitable for hard tissue repair.

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References

1. Splinter RJ, Hench LL, Allen WC, Greenlee TK. Bonding mechanism at the interface of ceramic prosthetic implants. J Biomed Mater Res. 1971;74:1478.
2. Xynos ID, Edgar AJ, Buttery LDK, Hench LL, Polak JM. Gene-expression profiling of human osteoblasts following treatment

with ionic products of bioglass 45S5 dissolution. J Biomed Mater Res. 2001;55:151.
3. Valerio P, Pereira MM, Goes AM, Leite MF. The effect of ionic products from bioactive glass dissolution on osteoblast proliferation and collagen production. Biomaterials. 2004;25:2941.
4. Sun JY, Wei L, Liu XY, Li JY, Li BE, Wang GC, et al. Influences of ionic dissolution products of dicalcium silicate coating on osteoblastic proliferation, differentiation and gene expression. Acta Biomater. 2009;5:1284.
5. Carlisle EM. Silicon: a possible factor in bone calcification. Science. 1970;167:279.
6. Carlisle EM. Silicon an essential element for chick. Fed Proc. 1972;31:A700.
7. Schwarz K, Milne DB. Growth promoting effects of silicon in rats. Nature. 1972;239:333.
8. Burch RE, Hahn HKJ, Sullivan JF. Newer aspects of the roles of zinc, manganese and copper in human nutrition. Clin Chem. 1975;21:501.
9. Parisi AF, Vallee BL. Zinc metalloenzymes: characteristics and significance in biology and medicine. Am J Clin Nutr. 1969; 22:1222.
10. Hsieh HS, Navia JM. Zinc deficiency and bone formation in guinea-pigs alveolar implants. J Nutr. 1980;110:1581.
11. Hurley LS. Teratogenic aspects of manganese, zinc, and copper nutrition. Physiol Rev. 1981;61:249.
12. Kim JT, Baek SH, Lee SH, Park EK, Kim EC, Kwun IS, et al. Zinc-deficient diet decreases fetal long bone growth through decreased bone matrix formation in mice. J Med Food. 2009; 12:118.
13. Yamaguchi M. Role of zinc in bone formation and bone resorption. J Trace Elem Exp Med. 1998;11:119.
14. Ito A, Ojima K, Naito H, Ichinose N, Tateishi T. Preparation, solubility, and cytocompatibility of zinc-releasing calcium phosphate ceramics. J Biomed Mater Res. 2000;50:178.
15. Sogo Y, Ito A, Fukasawa K, Sakurai T, Ichinose N. Zinc containing hydroxyaptite ceramics to pro-mote osteoblastic cell activity. Mater Sci Technol. 2004;20:1079.
16. Miao SD, Weng WJ, Cheng K, Du PY, Shen G, Han GR, et al. In vitro bioactivity and osteoblast-like cell test of zinc containing fluoridated hydroxyapatite films. J Mater Sci Mater Med. 2007;18:2101.
17. Lusvardi G, Zaffe D, Menabue L, Bertoldi C, Malavasi G, Consolo U. In vitro and in vivo behaviour of zinc-doped phosphosilicate glasses. Acta Biomater. 2009;5:419.
18. Du RL, Chang J, Ni SY, Zhai WY, Wang JY. Characterization and in vitro bioactivity of zinc-containing bioactive glass and glass-ceramics. J Biomater Appl. 2006;20:341.
19. Boyd D, Carroll G, Towler MR, Freeman C, Farthing P, Brook IM. Preliminary investigation of novel bone graft substitutes based on strontium–calcium–zinc–silicate glasses. J Mater Sci Mater Med. 2009;20:413.
20. Lin KL, Zhai WY, Ni SY, Chang J, Zeng Y, Qian WJ. Study of the mechanical property and in vitro biocompatibility of $CaSiO_3$ ceramics. Ceram Int. 2005;31:323.
21. Ni SY, Chou L, Chang J. Preparation and characterization of forsterite (Mg_2SiO_4) bioceramics. Ceram Int. 2007;33:83.
22. Wu CT, Chang J. A novel akermanite bioceramic: preparation and characteristics. J Biomater Appl. 2006;21:119.
23. Wu CT, Chang J, Zhai WY. A novel hardystonite bioceramic: preparation and characteristics. Ceram Int. 2005;31:27.
24. Ramaswamy Y, Wu CT, Zhou H, Zreiqat H. Biological response of human bone cells to zinc-modified Ca–Si-based ceramics. Acta Biomater. 2008;4:1487.
25. Wang HF, Ma YQ, Yi GS, Chen DP. Synthesis of Mn-doped Zn_2SiO_4 rodlike nanoparticles through hydrothermal method. Mater Chem Phys. 2003;82:414.
26. Cui HT, Zayat M, Levy D. Nanoparticle synthesis of willemite doped with cobalt ions ($Co_{0.05}Zn_{1.95}SiO_4$) by an epoxide-assisted sol-gel method. Chem Mater. 2005;17:5562.
27. Guo YP, Ohsato H, Kakimoto KI. Characterization and dielectric behavior of willemite and TiO_2-doped willemite ceramics at millimeter-wave frequency. J Eur Ceram Soc. 2006;26:1827.
28. Josset Y, Nasrallah F, Jallot E, Lorenzato M, Dufour-Mallet O, Balossier G, et al. Influence of physicochemical reactions of bioactive glass on the behavior and activity of human osteoblasts in vitro. J Biomed Mater Res A. 2003;67A:1205.
29. Hench LL. Bioceramics. J Am Ceram Soc. 1998;81:1705.
30. Knudsen FP. Dependence of mechanical strength of brittle polycrystalline specimens on porosity and grain size. J Am Ceram Soc. 1959;42:376.
31. Yu B, Liang KM, Gu SR. Effect of the microstructure on mechanical properties of CaO–P_2Os–MgO–F-glass ceramics. Ceram Int. 2003;29:695.
32. Yamaguchi M, Oishi H, Suketa Y. Stimulatory effect of zinc on bone formation in tissue culture. Biochem Pharmacol. 1987; 36:4007.
33. Yamaguchi M, Goto M, Uchiyama S, Nakagawa T. Effect of zinc on gene expression in osteoblastic MC3T3-E1 cells: enhancement of Runx2, OPG, and regucalcin mRNA expressions. Mol Cell Biochem. 2008;312:157.
34. Reffitt DM, Ogston N, Jugdaohsingh R, Cheung HFJ, Evans BAJ, Thompson RPH, et al. Bone density improves with disease remission in patients with inflammatory bowel disease. Bone. 2003;32:127.
35. Xynos ID, Edgar AJ, Buttery LDK, Hench LL, Polak JM. Ionic products of bioactive glass dissolution increase proliferation of human osteoblasts and induce insulin-like growth factor II mRNA expression and protein synthesis. Biochem Biophys Res Commun. 2000;276:461.
36. Wu CT, Chang J. Degradation, bioactivity, and cytocompatibility of diopside, akermanite, and bredigite ceramics. J Biomed Mater Res B. 2007;83B:153.