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Preparation and characteristics of a calcium magnesium silicate (bredigite) bioactive ceramic

Chengtie Wu[a,b], Jiang Chang[a,*], Junying Wang[c], Siyu Ni[a], Wanyin Zhai[a]

[a]*Biomaterials and Tissue Engineering Research Center, Shanghai Institute of Ceramics, Chinese Academy of Sciences, 1295 Dingxi Road, Shanghai 200050, People's Republic of China*
[b]*Graduate School of Chinese Academy of Sciences, People's Republic of China*
[c]*The School of Biology Science, East China Normal University, 3663 Zhongshan North Road, Shanghai 200062, People's Republic of China*

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Abstract

In this study, new bredigite ($Ca_7MgSi_4O_{16}$) ceramics were prepared by sintering sol–gel-derived bredigite powder compacts at 1350 °C for 8 h. The bending strength, fracture toughness and Young's modulus were about 156 MPa, 1.57 MPa $m^{1/2}$ and 43 GPa, respectively. The in vitro bioactivity of the bredigite ceramics was evaluated by investigating the apatite-formation ability in simulated body fluid (SBF) and the effect of ionic products from bredigite dissolution on the mouse fibroblasts cell line L929. In addition, the in vitro biocompatibility of the bredigite ceramics was evaluated by osteoblasts adhesion and proliferation assay. The results showed that bredigite ceramics could induce HAp formation in SBF. The products from bredigite dissolution significantly promoted cell growth at a certain concentration range. Furthermore, osteoblasts adhered and spread well on bredigite ceramics, and osteoblasts proliferation on bredigite ceramics was obvious.

Keywords: Bredigite; Bioactivity; Hydroxyapatite; Osteoblasts; Biocompatibility

1. Introduction

Previous studies showed that bioglass, glass-ceramic and bioceramics containing CaO and SiO_2 possessed good biocompatibility and bioactivity [1–8]. The basis of bioactive materials is the chemical reactivity of the materials in simulated body fluid (SBF) and HAp formation on the surface of materials. Ca and Si ions play an important role in the nucleation and growth of HAp, and influence the biological metabolism of osteoblastic cells essential in the mineralization process and bone-bonding mechanism [9]. Mg is also undoubtedly one of the most important elements in the human body. It is closely associated with mineralization of calcined tissues [10] and indirectly influences mineral metabolism [11]. Furthermore, diopside ($CaMgSi_2O_6$), a Ca, Si and Mg-containing ceramic, has been reported to possess apatite-formation ability in SBF and can closely bond to bone tissue when implanted in rabbits [6–8]. As an analogue with diopside in component, bredigite ($Ca_7MgSi_4O_{16}$) is also a Ca, Si and Mg-containing ceramic. Therefore, it is reasonable to assume that bredigite ceramics may possess apatite-formation ability.

In this study, the bredigite ceramics were prepared by sintering bredigite powder compacts, and the mechanical properties, bioactivity and biocompatibility of bredigite ceramics were evaluated.

2. Experimental

2.1. Preparation of bredigite ceramics

Bredigite powders were prepared by sol–gel process using tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS),

*Corresponding author. Tel.: +86 21 52412804; fax: +86 21 52413903.
E-mail address: jchang@mail.sic.ac.cn (J. Chang).

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magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$) and calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$). Bredigite ceramics were prepared by uniaxial pressing of the bredigite powders at 10 MPa followed by isostatic pressing at 200 MPa and sintering at 1350 °C for 8 h. To evaluate mechanical properties, the bars of bredigite ceramics with dimensions of $4 \times 8 \times 45.5$ mm were prepared. For the evaluation of the in vitro bioactivity and biocompatibility, ceramic discs with dimensions of 6 mm $\varnothing \times 2.5$ mm were also prepared by the same procedure.

2.2. Characterization of the bredigite ceramics

The sintered ceramics were analyzed by XRD and SEM. Bending strength and Young's modulus were applied normally with a deflectometer (AG-5KNL, Shimadzu Co., Japan). The fracture toughness was evaluated by a material testing machine (Instron 5566, USA) through the single notched precrack bending (SNPB) method [12]. The apparent density of bredigite ceramics was measured in water using the Archimedean technique.

2.3. Soaking bredigite ceramics in SBF

The ceramic discs polished by diamond paste were soaked in SBF (pH 7.25) at 37 °C for 6 h, 1, 3, 7, 10 and 20 days, and the ratio of disc surface area to solution volume of SBF was 0.1 cm^2/ml. The ion concentrations of SBF were similar to those in human blood plasma according to Kokubo [13]. After the set soaking time, the bredigite ceramic discs were characterized by XRD, SEM and an electron probe X-ray microanalyzer (EPMA-8705QH2, Shimazu, Japan) coupled with an energy-dispersive spectrometer (EDS, INCA Energy, Oxford Instruments, UK). The changes in elements concentrations in SBF were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Varian Co., USA), and the changes in pH were determined using an electrolyte-type pH meter (PHS-2C; Jingke Leici Co., Shanghai, China).

2.4. Effect of products from bredigite dissolution on mouse fibroblasts cell line L929

The method was carried out with a dilution of bredigite extract in contact with L929 cells. The ratio of the bredigite weight and the medium volume was 200 mg/ml. After incubation at 37 °C for 24 h, the mixture was centrifuged and the supernatant was collected. Subsequently, the extract liquid was diluted with serum-free medium for further cell culture experiments.

The cell suspension was adjusted to a density of 1×10^4 cells/ml, and 100 μl cell suspension was added to each well of a 96-well plate and incubated for 24 h. The culture medium was then removed and replaced by 50 μl of RPMI 1640 (Roswell Park Memorial Institute 1640) medium (Gibco, USA) supplemented with 20% FCS and 50 μl of diluted extracts (1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$). The medium supplemented with 10% FCS without the addition of diluted extracts was used as a negative control, and 50 μl of RPMI 1640 medium supplemented with 20% FCS and 50 μl solution of 0.2% Triton X-100 was used as a positive control. After cells were incubated at 37 °C and 5% CO_2 for 1, 3 and 7 days, 100 μl of 0.5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution was added in each well. After additional incubation for 4 h, dimethyl sulfoxide (DMSO) was added to stop the reaction between MTT and cells. The optical density (OD) was measured at the wavelength of 590 nm using an enzyme-linked immunoadsorbent assay (ELISA) plate reader (ELx 800, BIO-TEK, USA).

2.5. Osteoblasts adhesion and proliferation on the bredigite ceramics

Osteoblasts were isolated by sequential trypsin–collagenase digestion on calvaria of neonatal (<2 days old) Sprague–Dawley rats as described elsewhere [14]. In this study, only the cells at the 2nd–5th passage were employed. For evaluation of osteoblasts adhesion, the cells were seeded on each disc at a density of 8000 cells/cm^2. After culturing for 6 and 24 h, the discs were dyed with Giemsa solution, and observed by optical microscopy (Leica S6D, Germany). For SEM observation, the discs were dehydrated in a grade ethanol series (30%, 50%, 70%, 90% and 96% (v/v)) for 10 min, respectively, with final dehydration in absolute ethanol twice followed by drying in hexamethyldisilizane (HMDS) ethanol solution series [15]. For evaluation of osteoblasts proliferation, the cells were seeded at a density of 3500 cells/cm^2. After culturing for 1, 3 and 5 days, the discs were dyed with Giemsa solution, and counted under optical microscopy. Cell density (cells/cm^2) was determined by averaging the number of adherent cells in six randomly selected fields per substrate.

2.6. Statistical analysis

Six experiments were performed. The results are shown as the arithmetic means ± the standard deviation (±SD). Analysis of the results was carried out using Student's *t*-test, with a significance level of $p < 0.05$.