J Mater Sci: Mater Med (2008) 19:1257–1263
DOI 10.1007/s10856-007-3233-0

Synthesis and characteristics of monticellite bioactive ceramic

Xianchun Chen · Jun Ou · Yunqing Kang · Zhongbing Huang · Hongyang Zhu · Guangfu Yin · Haiming Wen

Received: 4 February 2007 / Accepted: 5 July 2007 / Published online: 16 August 2007

Abstract Mono-phase ceramics of monticellite ($CaMgSiO_4$) were successfully synthesized by sintering sol–gel-derived monticellite powder compacts at 1,480 °C for 6 h. The mechanical properties and the coefficient of thermal expansion (CTE) of the monticellite ceramics were tested. In addition, the bioactivity in vitro of the monticellite ceramics was evaluated by investigating their bone-like apatite-formation ability in simulated body fluid (SBF), and the biocompatibility in vitro was detected by osteoblast adhesion and proliferation assay. The results showed that the bending strength, fracture toughness and Young's modulus of the monticellite ceramics were about 159.7 MPa, 1.63 MPa $m^{1/2}$ and 51 GPa, respectively. The CTE was 10.76×10^{-6} °C^{-1} and close to that of Ti-6Al-4V alloy (10.03×10^{-6} °C^{-1}). Furthermore, the monticellite ceramics possessed bone-like apatite-formation ability in SBF and could release soluble ionic products to significantly stimulate cell growth and proliferation. In addition, osteoblasts adhered and spread well on the monticellite ceramics, which indicated good bioactivity and biocompatibility.

1 Introduction

During the last four decades, various bioactive materials such as sintered hydroxyapatite (HAp), 45S5 bioglasses, A/W glass-ceramics and so on, have been synthesized and developed for medical applications [1–8]. Sintered HAp is one of the most widely used calcium phosphate biomaterials due to its biological affinity. Because of the low fracture toughness and high Young's modulus, its application is generally restricted to non-load-bearing areas, unless combined with other materials offering better mechanical properties. Therefore, nearly all bone and tooth implants are presently made from metal alloys coated with HAp to improve the rate of implant fixation and prolong its longevity [8–10], and HAp-coated Ti-6Al-4V alloy has been accepted as one of the most promising implant materials [11]. The HAp coating, however, suffers from poor adhesion to the Ti-6Al-4V alloy substrate because of their large difference in coefficient of thermal expansions (CTE) [12], so it is necessary to synthesize a material having matchable CTE with Ti-6Al-4V alloy substrate.

A significant characteristic of bioactive materials is the chemical reactivity of the materials in simulated body fluid (SBF) and bone-like apatite formation on the surface of materials [4]. The proposed mechanism of bone-like apatite formation has been investigated by Siriphannon et al. [13], Liu et al. [14] and Wu et al. [15]. Recently, some papers reported that ionic dissolution products containing Ca- and Si- from bioactive glasses could stimulate osteoblast proliferation and gene expression [16–19]. In addition, it has been indicated that Mg ions could significantly enhance osteoblast adhesion [20] and directly stimulate osteoblast proliferation [21].

Therefore, in this study, monticellite ($CaMgSiO_4$) powders were synthesized by sol–gel method firstly, and then the single-phase ceramics of monticellite were successfully prepared by sintering monticellite powder compacts. The mechanical properties, the coefficient of thermal expansion (CTE), bioactivity and biocompatibility

X. Chen · J. Ou · Y. Kang · Z. Huang · H. Zhu · G. Yin (✉)
College of Materials Science and Engineering, Sichuan University, Chengdu, Sichuan 610064, P.R. China
e-mail: nic0700@scu.edu.cn

H. Wen
Shanghai Institute of Ceramics, Chinese Academy of Sciences, 1295 Dingxi Road, Shanghai 200050, P.R. China

of the monticellite ceramics were evaluated in vitro, and we discovered that compared with HAp, the monticellite ceramics had higher fracture toughness, lower Young's modulus, CTE closer to that of Ti-6Al-4V alloy and good bioactivity and biocompatibility in vitro.

2 Experimental procedure

2.1 Material preparation

Monticellite powders were synthesized by sol–gel process using tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS), magnesium nitrate hexahydrate ($Mg(NO_3)_2{\cdot}6H_2O$) and calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) as starting materials and HNO_3 as a precipitant. The monticellite ceramic wafers were prepared by uniaxial pressing of the monticellite powders under 20 MPa and sintering the compacts at 1,480 °C for 6 h. HAp powders were supplied by Research Center for Nano-Biomaterials, Sichuan University. The HAp ceramic wafers were prepared by uniaxial pressing of the HAp powders at 40 MPa and sintering at 1,100 °C for 6 h.

To evaluate mechanical properties and the CTE, the bars of the monticellite and HAp ceramics with dimensions of $3 \times 4{\times}36$ mm^3 and $4 \times 8{\times}10$ mm^3 were prepared, respectively. For the evaluation of the bioactivity and biocompatibility in vitro of the monticellite ceramics, ceramic wafers with dimensions of $\Phi 15 \times 3.5$ mm^2 were also synthesized by the same procedure.

The sintered HAp ceramics were identified by X-ray diffraction (XRD, X ′Pert MPD 3kW, Philips, Holand). The monticellite ceramics were identified by XRD and scanning electron microscopy (SEM, JSM-5900LV, Japan). Bending strength, fracture toughness and Young's modulus of the sintered ceramics were measured by an electronic universal machine (AG-10TA, Shimadzu, Japan). The coefficients of thermal expansion (CTE) were evaluated by a Dilatometer (Dll 402C, Netzsch, Germany). The apparent densities were measured in water using the Archimedean technique and the relative densities were calculated from the apparent and true densities [5, 22, 23].

2.2 Soaking the monticellite ceramics in SBF

The monticellite ceramic wafers were soaked in SBF solution (pH value = 7.40) at 37 °C for 15 and 30 days, and the ratio of wafer surface area to solution volume of SBF was 0.15 cm^2/mL. The SBF solution was prepared according to the procedure described by Kokubo [24].

After the pre-selected soaking time, the monticellite ceramic wafers were taken out, rinsed three times with double-distilled water and dried for 24 h at room temperature. Then they were characterized by SEM coupled with an energy-dispersive spectrometer (EDS) and thin-film X-ray diffraction (TF-XRD, X ′Pert MPD 3kW, Philips, Holand). Bone-like apatite formation on the ceramic surfaces was determined by fourier transform infrared spectroscopy (FTIR, Spectrum One, PerkinElmer, USA). The changes in element concentrations in SBF were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Jarrell-Ash ICAP9000, USA), and the changes in pH of the solution were identified using an electrolyte-type pH meter (PHS-3C, China).

2.3 Osteoblast culture

Osteoblasts were isolated by sequential trypsin–collagenase–hyaluronidase digestion on the shank of 3-month old Sprague-Dawley rats supplied by animal center of West China Medical School, Sichuan University. Briefly, the shank was dissected and cut into small pieces under aseptic conditions and rinsed several times with phosphate-buffered saline (PBS). To minimize fibroblastic contamination and cell debris, the shank pieces were incubated with 0.25% trypsin enzyme solution for 20 min, followed by five sequential digestions with 0.2% collagenase and 0.1% hyaluronidase in a metabolic shaker at 37 °C for 60 min each and discarded the first and second supernatant. After continuous enzyme treatment, the supernatant was centrifuged at 300*g* for 10 min, and the pellets were resuspended in Ham's F-12 culture medium supplemented with 10% fetal bovine serum (FBS) and maintained in a controlled humidified chamber at 37 °C and 5% CO_2. Media were changed every other day until the cells reached confluence. In this study, only the cells at the third–fifth passage were employed.

2.4 Osteoblast adhesion and proliferation on the monticellite ceramics

The osteoblasts were seeded on each opaque ceramic wafer at a density of 2.0×10^5 cells/cm^2 in a 24-well plate and incubated for 3 and 7 days in Ham's F-12 culture medium supplemented with 10% FBS maintained in a humidified chamber at 37 °C and 5% CO_2. The cells incubated in the culture medium without any additions were used as the control group (Blank).

After different culture time, the wafers were removed from the culture wells, rinsed with PBS and dehydrated in a graded ethanol series (30%, 50%, 70%, 90%, and 96% (v/v)) for 20 min, respectively, with final dehydration in absolute ethanol twice followed by drying in hexamethyldisilizane

(HMDS) ethanol solution series [4]. And then, the osteoblast morphology was observed by SEM. Cell's proliferation was evaluated by the quantitative MTT assay. In total, 100 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution was added in each well, followed by incubation for 4 h, at 37 °C. After incubation, 200 μL dimethyl sulfoxide (DMSO) was added in each well, and the samples were shaken until complete dissolution of the formed product. The optical density (OD) was measured at the wavelength of 570 nm using an enzyme-linked immunoadsorbent assay (ELISA) plate reader (EL×800, BIOTEK). The cell proliferation percentage was calculated with respect to the blank control.

2.5 Statistical analysis

The results are presented as Means ± SD (the numbers of the experiments are mentioned in the legend of the figure). Analysis of the results was carried out using ANOVA and Bonferroni's post-test. A p value < 0.05 was considered statistically significant.

3 Results

3.1 Characterization of the sintered ceramics

The XRD patterns of the sintered HAp and monticellite ceramics are shown in Figs. 1 and 2, respectively. Only HAp and monticellite peaks were detected by XRD. Figure 3 shows the SEM micrograph of the prepared monticellite ceramics. It could be seen that most monticellite particles had sintered, compact microcrystalline appearance with clear grain boundaries. The relative densities, mechanical properties and CTE of the monticellite and HAp are shown in Table 1. The relative density, bending strength, fracture toughness, Young's modulus and CTE of monticellite were about 94.1%, 159.7 MPa, 1.63 MPa $m^{1/2}$, 51 GPa and 10.76×10^{-6} °C^{-1}, respectively, while those of HAp were about 93.4%, 107.3 MPa, 0.86 MPa $m^{1/2}$, 67 GPa and 13.4×10^{-6} °C^{-1}, respectively.

3.2 Formation of bone-like apatite

Figure 4 shows the XRD pattern of the monticellite ceramics before soaking and the TF-XRD patterns of the monticellite ceramics soaked in SBF for 15 and 30 days. It was obvious that the intensity of monticellte peaks became lower and the characteristic peaks of bone-like apatite (JCPD 24-0033) appeared after soaking for 15 days (Fig. 4b). After 30 days of soaking, the intensity of bone-

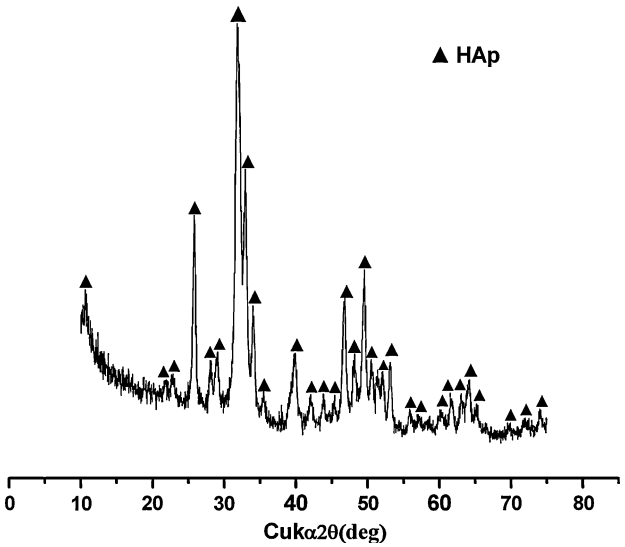

Fig. 1 XRD pattern of the sintered HAp ceramics

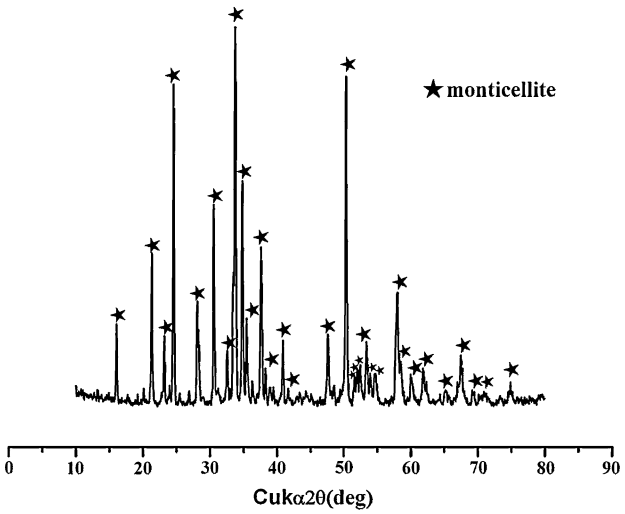

Fig. 2 XRD pattern of the synthesized monticellite ceramics

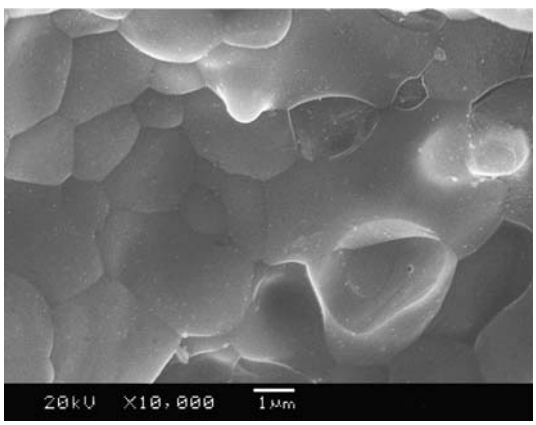

Fig. 3 SEM micrograph of the prepared monticellite ceramics

Table 1 Properties of the monticellte and HAp

	Monticellite	Hap
Composition (wt%)		
CaO	35.8	55.8
MgO	25.8	0
SiO_2	38.4	0
P_2O_5	0	42.4
Relative density (%)	94.1 ± 0.6	93.4 ± 0.9
Bending strength, MPa	159.7	107.3
Fracture toughness, MPa $m^{1/2}$	1.63	0.86
Young's modulus, GPa	51	67
CTE, $°C^{-1}$	10.76×10^{-6}	13.4×10^{-6}

like apatite peaks became higher and more obvious, while the peaks of monticellite disappeared (Fig. 4c).

The SEM micrographs of the monticellite ceramics soaked in SBF for various time periods are presented in Fig. 5. The surface of the monticellte ceramics was smooth before soaking (Fig. 3). In contrast, after soaking for 15 days, a layer of bone-like apatite crystals was formed on the surface of the monticellite ceramics and the particles were worm-like (Fig. 5a, b). When the samples were soaked for 30 days in SBF, the worm-like apatite was more obvious and the crystalline layers of bone-like apatite became more compact. The high magnification micrograph showed that the size of the crystallites was about 200–500 nm in length and 70 nm in diameter (Fig. 5c, d).

Figure 6 demonstrates the EDS analysis of a cross-section of the monticellite ceramics soaked in SBF for 30 days, showing that the presence of calcium and phosphorus in the layer. The Ca/P ratio of the Ca–P layer was about 1.68, which was close to that of the stoichiometric HAp.

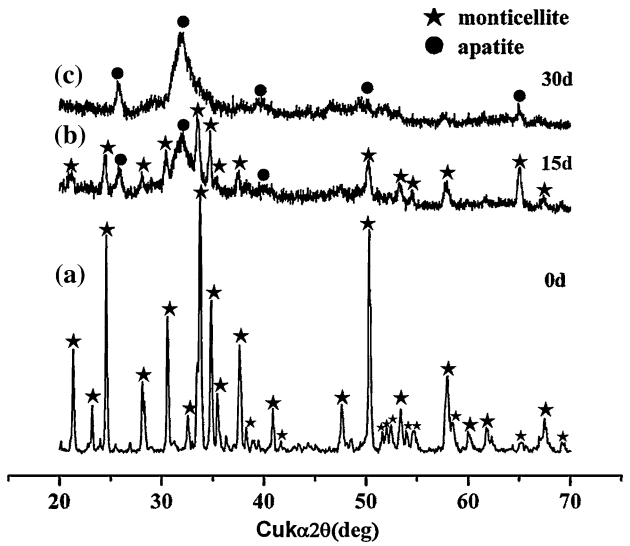

Fig. 4 XRD pattern of the monticellite ceramics before soaking and TF-XRD patterns of the monticellite ceramics soaked in SBF for 15 and 30 days. (a) 0 day; (b) 15 days; (c) 30 days

As shown in Fig. 7, the FTIR spectra of the surface product shows the characteristic bands of bone-like apatite at about 1,060, 961, 604, 567, 519 and 472 cm^{-1}. The absorption bands at about 1,640, 1,470, 1,424 and 879 cm^{-1} arising from the C–O and C=O vibrations at the positions of phosphate, were well defined in the samples [13]. In addition, absorption band of water at 3,460 cm^{-1} was also well defined. All these bands are similar to the typical FTIR bands of poorly crystallized HAp [25].

The changes in element concentrations in SBF and pH of SBF after soaking the monticellite ceramics for various time periods are presented in Fig. 8. The Ca, Mg and Si concentrations in SBF increased, while the concentration of P decreased with the increase of the soaking time. The pH of the SBF solution increased in the first 5 days of soaking and decreased after that.

3.3 Osteoblast adhesion and proliferation on the monticellite ceramics

The SEM micrographs show the morphological features of osteoblasts cultured on the monticellite ceramics for 3 and 7 days, respectively (Fig. 9). It was obvious that the osteoblasts attached on the surfaces of the monticellite ceramics after incubation, and exhibited an elongated and flattened appearance. After culturing for 7 days, the SEM micrograph more obviously showed that osteoblasts presented an elongated appearance, and minor filopodia could be observed (Fig. 9b).

Figure 10 displays the result of osteoblast proliferation on the monticellite ceramics after 3 and 7 days of culturing. In total, the OD values of osteoblasts were significantly higher than the blank control ($p < 0.05$) after being incubated for 3 and 7 days. After 7 days of incubation, the OD value of cells became higher, and the proliferation of osteoblasts was more obvious.

4 Discussion

In the present study, as shown in Table 1, a comparable bending strength, an improvement in the fracture toughness and a considerable decrease in the Young's modulus were achieved for the monticellite ceramics compared with sintered HAp. It was found that the monticellite ceramics might be more unlikely to fracture, and the Young's modulus of the monticellite ceramics was closer to those of the cortical bone (Young's modulus: 7–30 GPa) [6]. Furthermore, the CTE of the monticellite ceramics

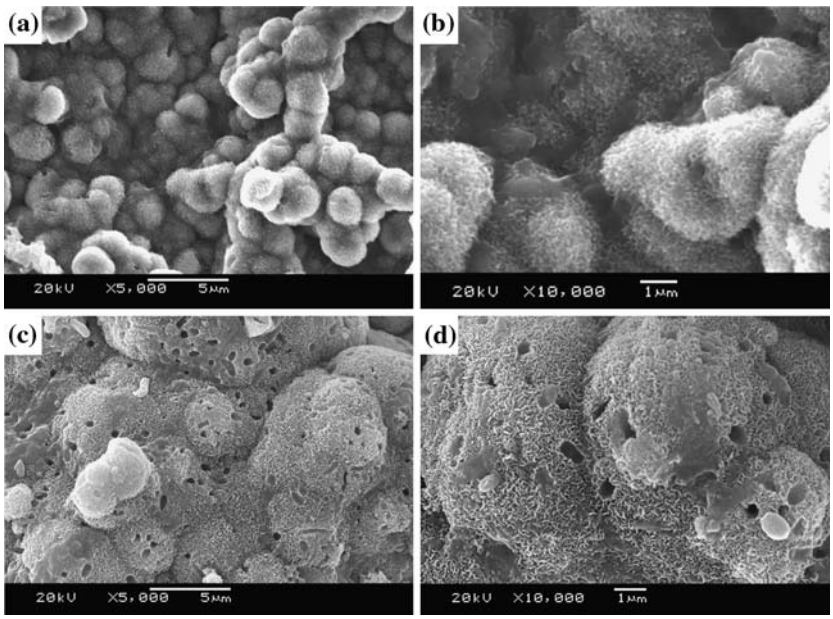

Fig. 5 SEM micrographs of the monticellite ceramics soaked in SBF for various time periods. (**a**, **b**) 15 days; (**c**, **d**) 30 days; (**b**, **d**) Higher-magnification photographs

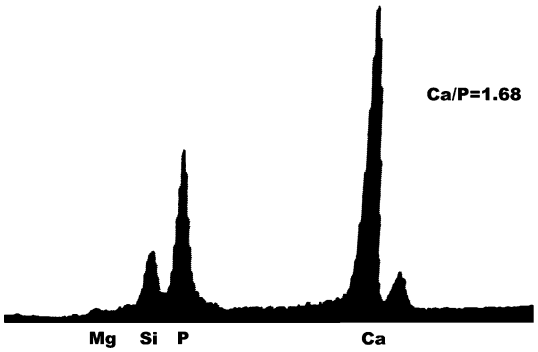

Fig. 6 EDS scanning analysis of a cross-section of the monticellite ceramics soaked in SBF for 30 days

(10.76×10^{-6} °C^{-1}) was closer to that of Ti-6Al-4V alloy (10.03×10^{-6} °C^{-1}) compared with HAp (13.4×10^{-6} °C^{-1}), which would contribute to a better match with Ti-6Al-4V alloy substrate when being used for coating material.

Previous studies showed bone-like apatite played an essential role in the formation, growth and maintenance of the tissue-biomaterial interface, and this bone-like apatite layer could be reproduced in SBF [4, 21]. Recently, some documents have reported that the akermanite ($Ca_2MgSi_2O_7$) and diopside ($CaMgSi_2O_6$) ceramics possessed strong bone-like apatite-formation ability in SBF and bone-like apatite with good bioactivity could be formed after some days of soaking [5, 15, 26, 27]. In this study, the

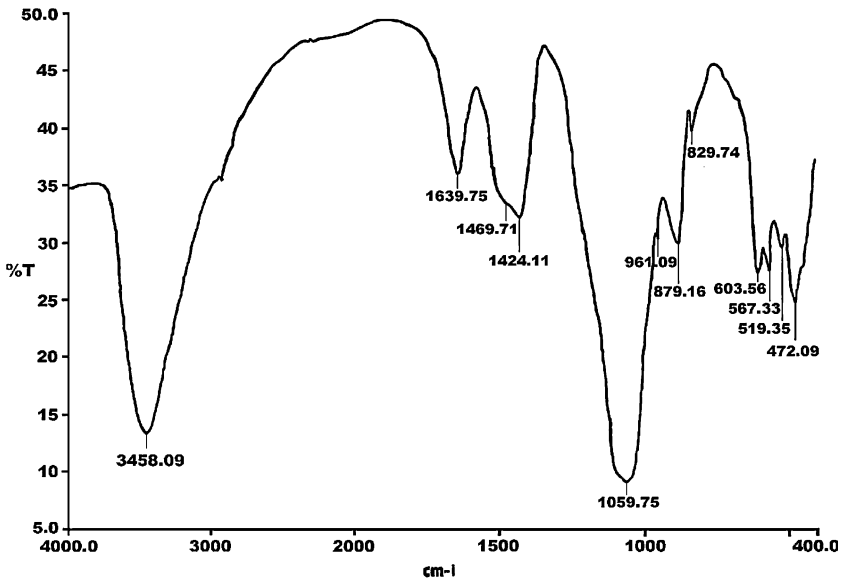

Fig. 7 FTIR spectra of the monticellite ceramic surface soaked in SBF showing bone-like apatite formation

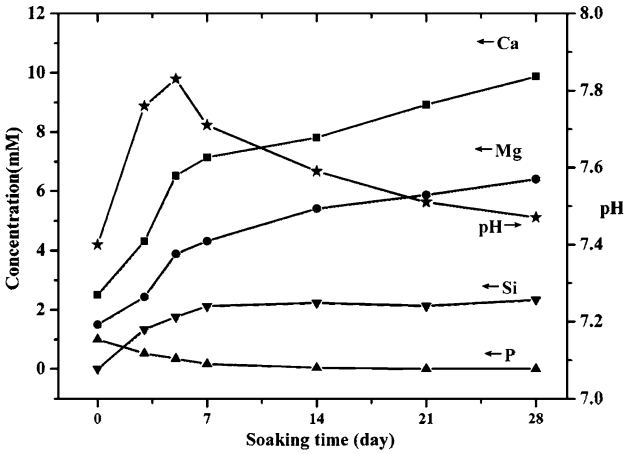

Fig. 8 Changes in ion concentrations and pH in the SBF solution after soaking the monticellite ceramics for various time periods

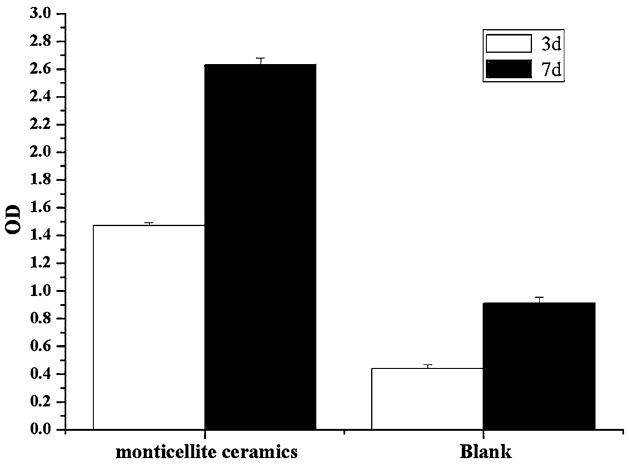

Fig. 10 The result of osteoblast proliferation on the monticellite ceramics after 3 and 7 days of culturing. Blank: blank control; OD on *y*-axis represents the number of the living osteoblasts

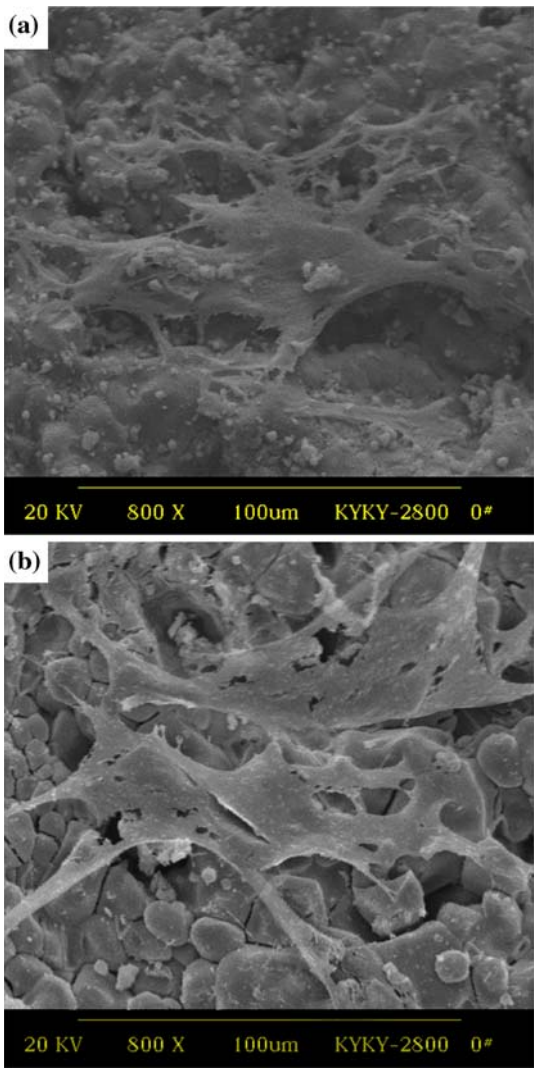

Fig. 9 SEM micrographs of the monticellite ceramics seeded with osteoblasts and cultured for different time periods. (**a**) 3 days; (**b**) 7 days

results indicated that the monticellite ceramics also possessed bone-like apatite-formation ability and were bioactive.

ICP-AES analysis detected that the Ca, Mg, and Si concentrations in SBF solution increased with the increase of soaking time. This phenomenon was attributed to the dissolution of the Ca, Mg, and Si ions from the monticellite ceramics. Although the formation of bone-like apatite layer consumed some Ca ions, the Ca ion dissolution from the monticellite ceramics was more than the Ca ion consumption. The decrease in P concentration was ascribed to the formation of amorphous calcium phosphate. The increase in pH of SBF at the early stage of soaking resulted from ion exchange of Ca^{2+} in the monticellite ceramics with H^{+} in SBF, which resulted in the formation of a hydrated silica layer providing favorable sites for phosphate nucleation on the surfaces of the ceramics, and the subsequent formation of bone-like apatite by incorporating OH^{-} ions from SBF conduced pH decrease with increasing soaking time [28]. The profile of the changes of Ca, Mg, Si and P concentrations, pH in SBF, and the formation of the silica-rich layer were similar to that of the bioactive akermanite and diopside ceramics [15, 26, 27]. All these results suggested that the mechanism of bone-like apatite formation on the surfaces of the monticellite ceramics might be similar to that of bone-like apatite formation on the bioactive akermanite and diopside ceramics.

Previous studies showed that Si- from bioactive glass dissolution promoted mineralized nodule formation of human primary osteoblasts and stimulated osteoblast proliferation and gene expression [16, 17, 19, 29]. Other studies showed that Ca ions could induce osteoblast proliferation and chemotaxis through binding to a G-protein coupled extracellular calcium sensing receptor [16, 30].

Monticellite is a Ca and Si-containing ternary ceramic, and the results showed that the ionic products from monticellite dissolution also promoted cell growth and the proliferation of osteoblasts adhering on the ceramics was more obvious with prolonged culture time, furthermore, osteoblasts adhered and spread well on the surfaces of the monticellite ceramics. These results indicated that the monticellite ceramics were bioactive and had osteoblast biocompatibility.

5 Conclusions

The monticellite ceramics were prepared by sintering monticellite powder compacts at 1,480 °C for 6 h. Compared with HAp, the bending strength and Young's modulus of the monticellite ceramics were closer to those of the cortical bone, and an improvement on the fracture toughness was achieved. The CTE of the monticellite ceramics was close to that of Ti-6Al-4V alloy, and they might be used for a bioactive coating on Ti-6Al-4V alloy substrate in the future. Furthermore, the monticellite ceramics could induce the formation of bone-like apatite layer on their surfaces when being soaked in SBF. The ion products from the monticellite ceramics significantly promoted cell growth and proliferation, and osteoblasts adhered and spread well on the surfaces of the monticellite ceramics. Our results indicated that the monticellite ceramics possessed high mechanical strength, an appropriate CET, good bioactivity and biocompatibility in vitro, and might be used as bioactive bone-tissue repair materials. Further studies in vivo are needed to explore the applicability of monticellite ceramics in the future.

Acknowledgment Financial supports from the Fund for Excellent Young Teachers of the Education Ministry of China (2002123) are gratefully acknowledged.

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