Research paper

Improvement of mechanical properties and biocompatibility of forsterite bioceramic addressed to bone tissue engineering materials

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ABSTRACT

This work deals with the fabrication and characterization of nanostructured forsterite bulk. This material may have better biocompatibility and mechanical properties than coarse grain forsterite for the development of bone tissue engineering materials. Nanostructured forsterite bulks were prepared by two step sintering of sol-gel derived forsterite nanopowder. Their sinterability and mechanical properties were then studied. Biocompatibility of the nanostructured forsterite bulk was also evaluated by cell attachment and proliferation experiments. In addition, the effects of ionic products from forsterite nanopowder dissolution on osteoblasts were studied. Results show that dense nanostructured forsterite bulk was prepared with hardness and fracture toughness of about 1102 Hv and 4.3 MPa m$^{1/2}$, respectively. Nanostructured forsterite was biocompatible and the MTT test confirmed that the products from forsterite nanopowder dissolution significantly promoted osteoblast proliferation within a certain concentration range. In addition, cells attached to and spread on the surface of nanostructured forsterite bulks. Mechanical properties of the nanostructured forsterite were much higher than that of hydroxyapatite. It was concluded that nanostructured forsterite is a bioactive ceramic with good biocompatibility that can be used as a bone tissue engineering material.

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1. Introduction

Bone tissue engineering proposes a suitable way to repair and regenerate lost bones. Different materials have been considered for use in bone tissue engineering (Chim et al., 2006). The biocompatibility of bioceramics makes them the most important group of biomaterials. Hydroxyapatite (HA) is a significant success of bioceramics as a bone tissue repairing material. HA is osteoconductive; a property that promotes bone tissue growth (Sprio et al., 2009). Weak mechanical properties such as low fracture toughness limit the clinical applications of HA (Hench, 1998). Dense HA has lower fracture toughness than cortical bone, and higher Young's modulus than cortical bone (Hench, 1998).

Forsterite (Mg$_2$SiO$_4$) is a new bioceramic. Previous research suggests that Mg is one of the most important elements in the human body. It indirectly influences the body mineral metabolism. Also, it is closely associated with mineralization of calcined tissues (Althoff et al., 1982). In addition, silicon is an essential element in skeletal development and is uniquely...
localized in the active areas of young bone. Silicon is involved in the early stage of bone calcification (Carlisle, 1970; Schwarz and Milne, 1972).

Research shows that forsterite has better mechanical properties than calcium phosphate ceramics such as HA (Ni et al., 2007). However, it is not mechanically strong for load bearing applications. Thus, improvement of mechanical features of forsterite is fundamental to reach equilibrium and reduce the mismatch to favor the osteointegration process. On the other hand, nanostructured ceramics have superior mechanical properties (Cottom and Mayo, 1996). In addition, the nanometer-sized grains and the high volume fraction of grain boundaries in nanostructured materials are reported to show improved biocompatibility over normal materials (Kim et al., 2008), and increased osteoblast adhesion and proliferation (Catledge et al., 2008; Du et al., 1999). Webster et al. (Webster et al., 1998) designed the first nanophase ceramics with the aim of improving osteointegrative properties of orthopedic and dental materials. In contrast to conventional materials, nanophase ceramics can be designed with surface properties, mechanical properties, and grain size distribution similar to natural bone (Gutwein and Webster, 2002).

According to the above points, nanostructured forsterite bioceramic is expected to have better mechanical properties and biocompatibility than coarser crystals. It was reported that coarse grain forsterite had an extremely low degradation rate and was not bioactive (Ni and Chou, 2008). Our recent study indicated that forsterite nanopowder, unlike micron-sized forsterite, possessed apatite-formation ability (Kharaziha and Fathi, 2009). The bioactivity of the nanophase forsterite when compared to coarse grain forsterite shows a greater effect of the nanophase forsterite on its ion dissolution in biological solution. As stated above, Si and Mg containing ionic products from the dissolution of forsterite nanopowder might have inhibitory effects on cell proliferation. Thus, an in vitro cytocompatibility evaluation of the nanostructured forsterite ceramics must be done.

In our previous research, the fabrication of dense nanostructured forsterite bulk with mechanical alloying derived forsterite nanopowder was studied (Fathi and Kharaziha, 2009). In this study, dense forsterite bulk was prepared by forsterite nanopowder derived via the sol-gel method (which has a smaller particle size and a narrow particle size distribution) to study the effects of these factors on the mechanical properties of the nanostructured forsterite dense ceramic. For the first time, an in vitro cytocompatibility evaluation of the nanostructured forsterite ceramics must be done. The effects of ionic products from forsterite nanopowder dissolution on an osteoblast were studied.

2. Experimental procedures

2.1. Preparation of forsterite ceramic

Forsterite nanopowder was prepared according to the modified sol-gel process described in our previous report (Kharaziha and Fathi, 2009). In brief, water-based solutions of the magnesium salts and colloidal silica were prepared.

An aqueous solution of sucrose was added to the solution. PVA solution was then added in to the final solution and the pH value was adjusted to 1. The solution was mixed homogeneously and heated at 80 °C for 2 h. The prepared gel was then heated at 100 °C in air for complete dehydration. In order to obtain the pure forsterite nanopowder, the dried gel was calcined in a furnace at 800 °C for 2 h (Kharaziha and Fathi, 2009). The X-ray diffraction pattern of forsterite nanopowder is shown in Fig. 1 (Kharaziha and Fathi, 2009). The crystallite size of forsterite powder according to Scherrer’s formula (Cullity, 1978) was in the range of 12–30 nm (Kharaziha and Fathi, 2009). Morphology and particle size of synthesized forsterite powder were studied by Transmission electron microscopy (TEM) technique.

Nanostructured forsterite ceramic bulks made of forsterite nanopowder were fabricated by sintering the green compacts of forsterite nanopowder. In order to break the foamy agglomerates, the as-synthesized nanopowder was ball-milled and mixed with a binder of 6 wt.% polyvinyl alcohol solution (the binder-to-powder ratio was optimized at 5/95 (w/w)). Green compacts with an approximate density of 54 ± 1.5% of the theoretical density (TD) were formed by uniaxially pressing the mixtures at 550 MPa in a cylindrical mould. Ceramic discs with dimensions of 11 mm Φ × 2.5 mm were prepared. To overcome the grain growth during the conventional sintering process, the sintering of the green bodies was performed with a two step sintering method. The two step sintering method (TSS), similar to the process which was described for forsterite nanopowder prepared by mechanical alloying method, in our previous research (Fathi and Kharaziha, 2009), was used. The cycle of two step sintering process was designed as follows:

(a) Heat the samples up to 600 °C and hold for 60 min at this temperature.
(b) Heat up to T1 = 900 °C and hold for 6 min at T1.
(c) Cool down to T2 = 750 or 850 °C and hold for 2–15 h at T2.

The heating rate of TSS was 10 °C min⁻¹. The cooling rate of TSS process, between T1 and T2, was 50 °C min⁻¹. After T2, it was 10 °C min⁻¹.

2.2. Characterization of the prepared ceramics

To estimate the crystallite size of the forsterite bulk, the bulks were crushed for XRD analysis. The crystallite size...
of the forsterite bulk was estimated from the XRD peak broadening based on Scherrer’s formula (Cullity, 1978). A scanning electron microscopy (SEM) was used to study the fracture surface of the forsterite ceramics. To accomplish this, the sintered specimens were mechanically ground and then polished. The grain size of the specimens was determined by multiplying the average linear intercept by 1.56 (Mendelson, 1969). For each specimen, 20 line segments were taken into account.

Linear shrinkage of the sintered samples was determined using the following equation (Eq. (1)):

\[
\text{Shrinkage(\%)} = \frac{l_g - l_p}{l_g} \times 100
\]

where \(l_g\) is the length of the green samples and \(l_p\) is the length of the sintered products.

Apparent density of the bulks was measured by Archimedes’ method. At least three sample sizes were used to determine the average grain size, shrinkage, and density corresponding to each data point.

The microhardness of each sample was tested using the Vickers indentation technique. This was done by applying a loading of 9.8 N for 15 s. Ten indentations were made on the polished surface perpendicular to the height direction. The fracture toughness was determined using the direct crack measurement method according to Niihara’s formula (Eq. (2)) (Niihara et al., 1982):

\[
K_{IC} = 0.203(c/a)^{-3/2}H_v a^{1/2}
\]

where \(K_{IC}\) is fracture toughness in MPa m\(^{1/2}\); \(c\) is the length of the crack measured from the center of the indentation at half of the average length of two indent diagonals; and \(H_v\) is the hardness.

2.3. Cytotoxicity assay

The method was carried out with a dilution of powder extract in contact with osteoblasts according to the International Standard Organization (ISO/EN 10993–5, 1999). A dilution of forsterite nanopowder extract in contact with osteoblast-like G292 Cells was used in the RPMI-1640 medium supplemented with 10% FCS (Fetal Calf serum). Prior to testing, the forsterite nanopowder were washed in 75% ethanol solution, sterilized for 20 min under ultraviolet light, and autoclaved for 30 min at 120 °C. After the discs were placed in a 24-well culture plate, 25 µL of culture medium containing 3 × 10\(^4\) cells were seeded onto the top of the discs. In order the cells to attach, the plate was incubated for 1 h. After, 1 ml of fresh culture medium was added to each well and the cells were incubated for 1–7 days. Culture media were refreshed every 2 days.

After incubation, the disks were washed with PBS solution and fixed for 30 min in 2.5% glutaraldehyde in phosphate-buffered solutions. The fixed cells were washed three times with PBS and dehydrated in varying concentrations of ethanol solution (30%, 50%, 70%, 90%, 95%, 100% (v/v)) for 10 min each. The discs were treated by immersion in the 50% alcohol-HMDS (hexamethyldisilazane) solution (v/v) for 10 min and then in pure HMDS for 10 min. Finally, they were dried in a desiccator overnight.

The cell morphology on nanostructured forsterite ceramics was observed with a scanning electron microscopy (SEM).

2.5. Statistical analysis

All data were collected with \(N = 3\) and expressed as means ± standard deviation (SD) in each experiment. Statistical analysis was done by two-way ANOVA with Duncan test. Differences were considered statistically significant at \(p < 0.05\).

3. Results and discussion

3.1. Characterization of forsterite ceramic

Fig. 2 shows the TEM micrograph of the forsterite nanopowder produced via sol–gel and mechanical alloying methods, respectively. As seen in Fig. 2(a, b), forsterite particles produced via the sol–gel method show a spherical shape with narrow particle size distribution in the 25–45 nm range (Kharaziha and Fathi, 2009). Fig. 2(c, d) show the TEM micrograph of forsterite nanopowder produced by mechanical alloying method (Fathi and Kharaziha, 2009). In contrast to the previous forsterite powder, the powder produced by mechanical alloying shows particles with
irregular shapes and with wide particle size distribution in the 25–70 nm range (Fathi and Kharaziha, 2009).

Fig. 3 shows the relative densities (RD) of the sintered samples as a function of $T_1$ (the first step sintering temperature) and grain size at two different second step temperatures (750 and 850 °C). As shown with this figure, by increasing the first step temperature up to 1000 °C, significant densification (about 79%–85%TD) was obtained in the first step of sintering at all temperatures (0 h-curve). Density improved from a lower value up to 98.6%TD in the second step of sintering. By increasing the holding time at the second step temperature, a fully dense ceramic was obtained (with $T_1 \geq 1200$ °C and a holding time of $T_2 \geq 5$ h) although grain growth occurred when $T_2 = 850$ °C.

Figs. 4 and 5 show the effect of the first step temperature of the TSS process on the fracture toughness and hardness values of forsterite ceramic. As can be observed, under this TSS regime ($T_1 = 1200$ °C and $T_2 = 750$ °C) with a prolonged soaking at 750 °C (up to 5 h), fracture toughness increased from $1.10 \pm 0.5$ MPa m$^{1/2}$ (at $T_1 = 900$ °C) to $4.3 \pm 0.3$ MPa m$^{1/2}$ (at $T_1 = 1200$ °C) and hardness increased from 520 ± 45 Hv (at $T_1 = 900$ °C) to 980 ± 20 Hv (at $T_1 = 1200$ °C). Under the TSS regime ($T_1 = 1200$ °C and $T_2 = 850$ °C) with a prolonged soaking at 850 °C up to 5 h, full density was observed.

The results also showed high hardness and fracture toughness of about 955 ± 15 Hv and 3.2 ± 0.25 MPa m$^{1/2}$, respectively. With prolonged soaking at 850 °C up to 15 h, hardness and fracture toughness declined.

Wang et al. (2006) suggests that for achieving densification without a significant grain growth, grain boundary diffusion needs to remain active, while the grain boundary migration is suppressed. A mechanism to slow down the grain boundary movement is the triple-point drag. Grain growth entails a competition between grain boundary mobility and junction mobility. Once the latter decreases, particularly at low temperatures in which junctions are rather immobile, the drag will occur. The grain growth prohibition is, therefore, achievable under the above circumstances. For the forsterite ceramic, $T_2 = 850$ °C might be too high to immobilize the
Fig. 6 – SEM micrograph of the fracture surface of forsterite with $T_1 = 1200 \, ^{\circ}\mathrm{C}$, $T_2 = 750 \, ^{\circ}\mathrm{C}$ (5 h).

table

Table 1 – Physical structure and mechanical properties of the nanostructured forsterite bulk obtained at $T_1 = 1200 \, ^{\circ}\mathrm{C}$ and $T_2 = 750 \, ^{\circ}\mathrm{C}$ in different second step holding times of the TSS process.

<table>
<thead>
<tr>
<th>Sintering time (h)</th>
<th>Relative density (% TD)</th>
<th>Shrinkage (%)</th>
<th>Crystallite size (nm)</th>
<th>Hardness (Hv)</th>
<th>Fracture toughness (MPa m$^{1/2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>83.1 ± 4.2</td>
<td>10.7 ± 3.2</td>
<td>20–22</td>
<td>800 ± 55</td>
<td>1.86 ± 0.21</td>
</tr>
<tr>
<td>5</td>
<td>98.6 ± 0.22</td>
<td>32.1 ± 1.8</td>
<td>30–45</td>
<td>1102 ± 25</td>
<td>4.3 ± 0.19</td>
</tr>
<tr>
<td>15</td>
<td>98.6 ± 0.13</td>
<td>33.4 ± 0.3</td>
<td>48–57</td>
<td>1092 ± 18</td>
<td>3.98 ± 0.25</td>
</tr>
</tbody>
</table>

grain boundary. The best condition for the TSS processing of the forsterite nanopowder is the TSS1 regime ($T_1 = 1200 \, ^{\circ}\mathrm{C}$ and $T_2 = 750 \, ^{\circ}\mathrm{C}$) with soaking at $750 \, ^{\circ}\mathrm{C}$ up to 5 h. Also, the presence of a lot of pores between grains when the first step temperature was lower than $1200 \, ^{\circ}\mathrm{C}$ and the second step temperature was $750 \, ^{\circ}\mathrm{C}$ resulted in grain growth. At lower temperatures the grain growth was significant.

Fig. 6 shows the SEM micrograph of the fracture surface of forsterite ceramic after the densification process under $T_1 = 1200 \, ^{\circ}\mathrm{C}$ and $T_2 = 750 \, ^{\circ}\mathrm{C}$ with a 5 h holding time at TSS second step. The micrograph of fracture surface of forsterite shows intergranular fracture, uniform grain size distribution in the 120–150 nm range without any observable cracks or pores and no abnormal grain growth. Also, well-defined grains are visible with almost negligible porosity.

Table 1 shows the results of relative density, linear shrinkage, crystallite size, hardness, and fracture toughness values obtained at $T_1 = 1200 \, ^{\circ}\mathrm{C}$ and $T_2 = 750 \, ^{\circ}\mathrm{C}$ at different second step sintering times of the TSS process. By increasing the second step sintering time of the TSS process from 2 to 15 h, the linear shrinkage and relative density of sintered forsterite ceramic bulks increased from 10.7% to 33.4% and from 83.1% to 98.6% TD, respectively. According to the results of Table 1, prolonged soaking of up to 5 h, gives the maximum values of hardness and fracture toughness which are about 1102 Hv and 4.3 MPa m$^{1/2}$, respectively.

In our previous work, nanostructured forsterite bulk was prepared by two step sintering of the mechanical alloying derived forsterite nanopowder (Kharaziha and Fathi, 2009). The improved mechanical properties at a lower sintering temperature were obtained in the present study. According to the TEM micrograph of forsterite nanopowder prepared via sol–gel and mechanical alloying processes, the smaller particles and narrow particle size distribution of forsterite prepared via the sol–gel method could induce better compatibility and sinterability. In addition, because of lower residual pores and smaller grain size in the sintered bulk, mechanical properties could be modified.

Ni et al. (2007) prepared fully dense forsterite ceramic from coarse grain forsterite powder with a relative density of about 92.5% TD by uniaxially pressing at 10 MPa, then cold isostatic pressing at 200 MPa. Sintering was done at 1450 °C with an 8 h holding time. The maximum $K_{IC}$ value for forsterite reported in this report was 2.4 MPa m$^{1/2}$ (Ni et al., 2007). Thus, the higher value of fracture toughness obtained in the present work was encouraging. This improvement in fracture toughness could be attributed to both the improved properties of the synthesized forsterite powder, as well as the improved sinterability of these powders with the limited grain growth obtained through TSS method.

It is well known that nanostructured materials have a higher driving force for densification than coarse grain powders which improves their sinterability (Edelstein and Cammarata, 1996). A comparison of the density values attained here with the results of the studies involving normal sintering indicates that the two step sintered nanoceramics reached higher density values, thus affecting their mechanical properties. However, the accelerated grain growth is reported in many studied cases (Han et al., 2007; Mazaheri et al., 2007; Ghosh et al., 2007; Yu et al., 2007), showing that the two step schedule could control the grain growth in the nanostructured materials. Two step sintering is very different from the conventional sintering methods.

Usually, in the normal sintering method, green compacts are heated in a first cycle at a predetermined rate, and held at a desired temperature until the highest densification level is reached. The grain size increases continuously as density increases which reduces the fracture toughness value. Whereas in the two step sintering methods, green compacts are heated up to a high temperature and held for a short time to reduce the pore sizes in the subcritical scale, then cooled to the lower temperature in order to complete the sintering process.

To the best of our knowledge, it is the first time that fully dense nanostructured forsterite ceramic with such a small grain size (in about 120–150 nm) and good mechanical properties ($K_{IC} = 4.3 \, \text{MPa m}^{1/2}$) is achieved. As can be seen, nanostructured forsterite ceramic has better mechanical properties than calcium phosphate ceramics, such as hydroxyapatite ceramics ($K_{IC} = 0.75–1.2 \, \text{MPa m}^{1/2}$) (Ni et al., 2007). In addition, it is bioactive. Thus, it might be a good replacement for HA ceramic as a bone tissue.
engineering material. Compared to other bioactive ceramics containing SiO$_2$, such as diopside (CaMgSi$_2$O$_6$) (Nonami and Tsutsumi, 1999), akermanite (Ca$_2$MgSi$_2$O$_7$) (Wu et al., 2006) and bredigite (Ca$_7$MgSi$_4$O$_{16}$) ceramics (Wu et al., 2005), nanostructured forsterite possessed significantly improved mechanical properties, which are some of the most important properties in the bone repairing ceramics.

Because of the higher surface energy in the nanostructured materials than the coarse grain samples, nanostructured forsterite might release inordinate ions in biological conditions. This could have diverse effects on the bone cells. Hence, we studied the in vitro biocompatibility of nanostructured forsterite.

3.2 Cytotoxicity assay

Fig. 7 shows the results of the MTT assay of forsterite nanopowder dissolution after different times of cell culturing. The results reveal that after 1 day of incubation, significant differences could not be detected among different concentrations of the forsterite nanopowder. Cells proliferated continually with increasing culture time in all samples. In the high concentrations of forsterite (50–200 mg/ml), cell numbers increased slowly. In contrast, in the low concentrations of forsterite (from 6.25 to 50 mg/ml), cells proliferated more actively compared to the other samples ($p < 0.05$). By culturing up to 7 days, the OD values of samples were significantly higher than the negative control ($p < 0.05$). The higher proliferation rate of samples having forsterite extracts than the negative control confirmed that forsterite promoted the proliferation of cells without a cytotoxic effect. Recent studies have demonstrated the positive stimulatory effects of extracellular Ca, Si, and Mg containing ionic products from the dissolution of MgO–SiO$_2$–CaO ceramic systems at a certain concentration range on the osteoblast-like cells (Wu et al., 2005). In summary, in spite of the important role of Mg ion dissolution in the bioactivity of inorganic biomaterials, at high concentration of extracts, the inhibitory effect on cell proliferation might be observed (Wu et al., 2005).

Based on the above results, although ionic products from forsterite nanopowder dissolution could stimulate cell proliferation only at a certain concentration range, inhibitory effects on cell growth were not seen in other concentration ranges. Ni et al. (2007) studied the biocompatibility of coarse grain forsterite by MTT testing. They showed that although forsterite is biocompatible, it did not show any significant difference between forsterite samples and control samples even in the longest period of cell culture. It showed that although micron size forsterite is biocompatible, it could not promote proliferation of cells.

According to the above results, by reducing the grain size to the nanoscale, forsterite bioceramic became bioactive and promoted cell proliferation in a concentration range of forsterite nanopowder.

3.3 Cell adhesion and growth assay

SEM images of the G292 cells cultured for 1–7 days on the nanostructured forsterite bulks are presented in Fig. 8. After 1 day, cells attached onto the ceramic and minor filopodia was observed (Fig. 8a). After 3 days, all of the cells adhered tightly to the sample surface and spread well (Fig. 8b). After 7 days, the cells formed a flattened sheet and the surface of all the samples were completely covered by the cells and the extracellular matrix (Fig. 8c). The attachment and spreading
of the cells on biomaterials are important processes of the cell/material interactions (Kirpatrick et al., 1997). Our results showed that G292 cells completely attached to and spread on the nanostructured forsterite ceramics.

Results indicate that nanostructured forsterite bioceramic possesses good bioactivity, biocompatibility and mechanical properties that make it especially suitable as a bone tissue repairing material. In our study, ionic concentrations of different samples showed obvious differences during cell culture. This indicates that it is possible to modulate the release rates of active ions (Mg and Si) by making a composite of forsterite nanopowder with other ceramics such as bioactive glass, Hydroxyapatite, or polymers to obtain the best desirable properties (i.e., mechanical properties and biocompatibility). Based on the results, the forthcoming study for the present authors is the in vivo evaluation of prepared bioactive forsterite.

4. Conclusion

Nanostructured forsterite (Mg$_2$SiO$_4$) bulks, made of forsterite nanopowder (grainsize = 25–45 nm) produced via sol-gel method, with grain size of 120–150 nm and crystallite size of 30–45 nm, was prepared with a two step sintering method. Their sintering behavior and mechanical properties were studied. Hardness (1102 Hv) and fracture toughness (4.3 MPa m$^{1/2}$) of nanostructured forsterite ceramics were higher than those of the currently available hydroxyapatite and coarse grain forsterite ceramics. The products from forsterite nanopowder dissolution significantly promoted cell proliferations at a certain concentration range. In addition, the in vitro study showed significant G292 cell adhesion, spread, and growth on the surface of the nanostructured forsterite ceramic. Results indicated that nanostructured forsterite bioceramic possesses good in vitro bioactivity, biocompatibility, and mechanical properties and can be used as a bioactive bone tissue engineering material. However, further in vivo studies need to be conducted to explore the applicability of this ceramic as a bone tissue material.

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