Ultra-fast, highly efficient and green synthesis of bioactive forsterite nanopowder via microwave irradiation

Mehdi Kheradmandfarda,b,⁎, Seyed Farshid Kashani-Bozorgb, Amir Hosein Noori-Alfesharakib,c,⁎, Anoushe Zargar Kharazic,d, Mansooreh Kheradmandfarda, Narges Abutalebf

a School of Metallurgy and Materials Engineering, College of Engineering, University of Tehran, Tehran, Iran
b Department of Materials Engineering, Tarbiat Modares University, Tehran, Iran
c Faculty of Biomaterials, Tissue Engineering and Nanotechnology, School of Advanced Technologies in Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
d Department of Chemistry, Isfahan University of Technology, Isfahan, Iran
e Department of Materials Engineering, Tarbiat Modares University, Tehran, Iran
f Biomaterials Research Group, Isfahan University of Medical Sciences, Isfahan, Iran

ARTICLE INFO

Keywords:
Forsterite
Nanopowder
Green synthesis
Microwave irradiation
Bioactivity
Cell culture

ABSTRACT

Forsterite (Mg2SiO4) has recently attracted considerable attention in different fields because of its wide range of applications. In this paper, pure forsterite nanopowders were synthesized by an ultra-fast, highly efficient and green method for the first time. Microwave irradiation was used to synthesize forsterite nanopowder. The formation of highly crystalline forsterite nanopowder was confirmed by X-ray diffraction (XRD) and energy dispersive X-ray spectrometer (EDS) analyses. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analyses showed that the agglomerated powder composed of nanocrystalline particles with the mean particle size of ~100 nm. Microwave irradiation significantly accelerated the rate of the reactions and dramatically decreased reaction times from hours to minutes and seconds. In vitro bioactivity evaluation was performed by soaking the forsterite samples in simulated body fluid (SBF). Results indicated that synthesized forsterite nanopowder via microwave irradiation method possessed excellent apatite-forming ability in SBF. Cell viability results showed that synthesized forsterite nanopowder not only showed no cytotoxicity but also improved cell proliferation. Alkaline phosphatase (ALP) activity assay indicated that the fabricated forsterite nanopowder could facilitate the MG63 osteoblast-like cells to proliferate and differentiate. Therefore, microwave-assisted synthesis technique could be considered as a novel, safe and high efficient method in saving time and energy for bioactive forsterite nanopowder production.

1. Introduction

Forsterite (Mg2SiO4) has attracted the attention of many scientists of different fields in the recent decades because of its wide range of applications [1–6]. Unique properties of forsterite such as high-quality factor value (~241,500 GHz), low dielectric constant (εr = 6.8) [3], chemical stability, excellent insulation properties even at high temperatures [2], good biocompatibility [4], and high mechanical properties make it as appropriate candidate for different applications such as dielectric substrates [5], refractory materials [6], solid oxide fuel cells [7], optical devices [8], and biomaterials [4].

It is well known that scaling down of the materials to the nano-scale may remarkably modify their chemical and physical properties. Thus, the advanced nanostructured materials usually exhibit an excellent combination of mechanical, chemical, physical, and magnetic properties compared to the micron-scale ones [9–11]. These excellent properties of nanostructured materials make them suitable candidates for applications in various fields. Therefore, bringing forsterite to the nano scale can improve substantially its properties such as biocompatibility, biodegradation, etc. [12].

Forsterite has been fabricated by various methods such as sol–gel [1, 2], polymer precursor [13], ball milling [14, 15], chemical vapor deposition [3], etc. However, it still remains a big challenge to synthesize pure forsterite nanoparticles through a cost effective and easy to optimize method. It has been reported that it is difficult to avoid the formation of enstatite (MgSiO3) and periclase (MgO) during the synthesis of forsterite by conventional methods and therefore, a high temperature of about 1200–1600 °C should be performed to obtain pure forsterite [16]. Furthermore, conventional methods usually need a long time to produce pure forsterite nanopowder. Hence, there is a strong
motivation to find a suitable method for the synthesis of pure forsterite nanopowder.

Very recently, there has been a major surge in employing microwave-based ultrafast, energy-efficient, facile, and “Green Chemistry” approach for synthesizing multi-component nanostructures including metals [17], metal oxides [18], metal sulfides [19], intermetallics [20], polymers [21], and ceramics [22] and their composites [23]. There are two unique motivations for using microwaves in different fields. The former is efficient internal heating produced by microwave irradiation, delivering energy exactly where needed. The latter is dramatic reduction in reaction times: from days and hours to minutes and seconds [24]. Several works have been reported on the fast synthesis of nanostructures materials [25–27]. Microwave-assisted synthesis of hydroxyapatite nanopowder was found to be significantly efficient in saving time and energy [25]. Zhou et al. [18] reported successful synthesis of Co3O4 quantum dots/graphene composites by a facile and efficient microwave irradiation method. Microwaved-assisted fast synthesis of n and p-doped MgSi has been reported by Bertheaud et al. [26]. SnTe nanoparticles were prepared via a simple and ultra-fast microwave hydrothermal method [27]. Hamedani et al. [28] has reported fabrication of ZnO nanocrystals with various morphologies via a fast and facile microwave-assisted method.

Keeping the above points in view, it is expected that synthesizing forsterite nanopowder by microwave-assisted method offers various advantages including rapid heating, shorter synthesis time, efficient energy transformation and pure product. In this study, an ultra-fast, highly efficient and green method was developed to prepare bioactive forsterite nanopowder for the first time.

2. Materials and methods

2.1. Powder preparation

Magnesium nitrate hexahydrate (Mg(NO₃)₂·6H₂O; Merck) and tetra ethyl ortho-silicate (TEOS) (Acron Organics, USA) were used as starting materials and silicon precursors. A designated amount of Mg(NO₃)₂·6H₂O (0.1 M) was dissolved in absolute ethanol. Then, a designated amount of TEOS (0.05 M) was added. The pH of solution was adjusted to 9 by adding NH₄OH and stirring was prolonged for 20 min at room temperature to form homogenous solution. The mixture was refluxed into a modified microwave oven (Samsung; MW71B) at 850 W and 2.45 GHz, and subjected to microwave irradiation under ambient conditions. The mixture was cooled to room temperature, the precipitated product was isolated by centrifugation, washed thoroughly with deionized water three times, and dried in an oven at 90 °C for 1 h. The obtained white colored powder served as the forsterite precursor that heat treated at a rate of 10 °C/min up to 800 °C for 2 h.

2.2. Powder characterization

Phase analysis of the synthesized powders was performed by a Philips diffractometer (40 kV) using Cu-Kα radiation (λ = 0.15406 nm) over the 2θ-range of 20–80° (the time per step and step size were 1 s and 0.02°, respectively). A field emission scanning electron microscope (FE-SEM, Hitachi S-4200) was utilized to investigate the morphology and size distribution of the synthesized powders. Energy dispersive X-ray spectrometry (EDS) analysis was carried out with a high-resolution scanning electron microscopy (HR-SEM) equipped with an EDS analyzer to measure elemental composition of the synthesized forsterite. Furthermore, the morphology and particle size distribution of the powders were investigated by transmission electron microscopy (TEM, Philips 208S) under an accelerating voltage of 200 kV. In this regard, the particle size of powders was estimated by Image J software.

2.3. In vitro biological studies

For in vitro biological studies, the forsterite discs were prepared using uniaxial pressure apparatus under 250 MPa pressure followed by two step sintering as reported by Fathi et al. [29] to retain nanostructured forsterite. Briefly, after pressing, the discs of 7 mm-diameter and 2.5 mm-thickness were placed in a programmable furnace. The samples were heated from ambient temperature to 1000 °C with a 5 °C/min rate and maintained for 6 min in this temperature. Then they were cooled to 750 °C with a 5 °C/min rate and maintained for 7 h in this temperature and finally cooled to environment temperature with a rate of 5 °C/min [29].

2.3.1. In vitro bioactivity evaluation

The formation of apatite layer on the surface of the forsterite discs was considered after immersing the discs in simulated body fluid (SBF). The synthesizing process of SBF was previously described by Kokubo [30]. The discs were soaked in SBF at 37 °C for 14, 21 and 28 days. After the preselected immersion time, the discs were removed from the SBF, washed with distilled water, and dried in an oven at 80 °C for 24 h; they were then subjected to SEM and EDS analyses. All the reacted solutions were kept for inductively coupled plasma optical emission spectrometry (ICP-OES) (OPTIMA 7300DV) to determine Ca, Mg, Si, and P ionic concentration in the SBF solution.

2.3.2. Cell viability study

The biological performance of nano-structured forsterite made by microwave-assisted method, was evaluated through osteoblast-like MG63, a human osteosarcoma cell line. For cell culture process, discs were placed into 24-well plates and sterilized by UV radiation for 2 h followed by immersion in 70% ethanol for another 2 h. Then a cell density of 10⁵ cells/cm² from MG63 osteoblast-like cells was suspended in 200 μl of cell culture medium and seeded on the forsterite discs as well as on tissue culture plate (TCP) as control group. The samples were incubated with MTT solution for 4 h. The dark blue formazan crystals were solubilized with dimethyl sulfoxide (DMSO, Sigma) as the MTT solvent and kept for 30 min at 37 °C. For each sample, 100 μl of the solution was moved to a 96-well plate and its absorbance was read by ELISA plate reader (Hyperion, Florida, USA) at 540 nm. The amount of absorption is proportional to the number of viable cells attached to each day. Three samples at each time zone were selected to study cell viability by MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT, Sigma-Aldrich)) assay. At the predicted times of culture (1, 3 and 5 days), the medium was discarded, and the samples were incubated with MTT solution for 4 h. The dark blue formazan crystals served as the forsterite precursor that heat treated at a rate of 10 °C/min up to 800 °C for 2 h.

2.3.3. Alkaline phosphatase (ALP) activity

ALP enzyme activity of cultured MG63 cell was performed using ALP assay kit (Pishatix Teb, Iran). Cell density of 10⁶ cells/cm² from MG63 cells were cultured for 7 and 14 days on nano-forsterite discs as well as TCP (as control). After the preselected times, cell culture medium was extracted and cultured cells were washed with PBS, then they were resuspended in 150 ml lysis buffer provided in kit. Then, samples were centrifuged at 12,000 rpm to remove any insoluble material and the solution was moved to the tubes. Finally, ALP activity was measured according to manufacturer’s instruction using ALP kits. The test kit works by transformation of colorless p-nitrophenyl phosphate (p-NPP) to colored p-nitrophenol (p-NP) and its absorbance measured spectrophotometrically using ELISA plate reader (Hyperion, Florida, USA).
USA) at 405 nm.

2.3.4. Statistical analysis

Results were evaluated using one-way analysis of variance (ANOVA) with three replicates and SPSS software (V16.0) and reported as mean ± standard deviation (SD). p-value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Powder characterization

Fig. 1 shows the XRD patterns of the calcined powder at 800 °C for 2 h. Forsterite was formed at 800 °C and no other peaks except for characteristic forsterite peaks were observed. Moreover, the presence of all sharp peaks in the XRD pattern confirms the formation of highly crystalline forsterite. Fig. 2 shows the morphology of the synthesized forsterite powders. It is apparent that the synthesized forsterite powders were composed of nearly similar particle size distribution and regular shape. FE-SEM image analysis (Fig. 2b) confirmed that the synthesized forsterite powders were composed of nanoparticles ranging from 50 to 100 nm.

EDS analysis of the obtained powder (Fig. 3) was found to be consistent with the XRD result; the spectrum consisted of oxygen (Kα ~ 0.53 keV), magnesium (Kα ~ 1.25 keV) and silicon (Kα ~ 1.74 keV) peaks and their quantitative ratios exhibited values that were somewhat close to those of forsterite stoichiometry (i.e., 2:1:4 for Mg₂SiO₄ as Mg₃SiO₄). However, oxygen appeared somewhat lower than that of forsterite stoichiometry; this is conventionally expected using EDS technique and related to the absorption of its low-energy X-ray. The morphological shape and size of the synthesized forsterite powder obtained from TEM is shown in Fig. 4. Furthermore, particle size histogram (Fig. 4b) indicated that the distribution of particle size was in the range of 50–130 nm. It can be seen from the Fig. 4b that the mean particle size of the synthesized forsterite powder was ~100 nm.

3.2. Forsterite nanopowder formation mechanism

The formation mechanism of forsterite nanopowder by microwave
radiation is schematically represented in Fig. 5. The initial step (a) for forsterite formation is the hydrolysis of TEOS. During the hydrolysis reaction of TEOS, its ethoxy group reacts with the H₂O molecule to form the intermediate [Si (OC₂H₅)₄−X (OH)X] with hydroxyl group replacing ethoxy groups. Ammonia has a basic catalyst role in this reaction; the hydrolysis reaction is started by the attacks of hydroxyl anions on TEOS molecules [31]. The aqueous ammonia solution plays as both the reactant (H₂O) and the catalyst (NH₃) for the hydrolysis of TEOS [32]. In the next step (b) Si(OH)₄ converts to silicate (SiO₄⁴⁻). In the final step (c), strikes of Mg²⁺ and SiO₄⁴⁻ ions result in the nucleation of forsterite. It is clear that the microwave irradiation significantly accelerated the rate of the reactions in each step. Indeed, microwave irradiation dramatically decreased reaction times: from hours to minutes and seconds.

Fathi et al. [14] reported a mechanical activation route (for 10 h) assisted with heat treatment at 1200 °C to obtain pure forsterite nanopowder. Bafrooei et al. [33] showed that 40 h ball milling and subsequent microwave heating at 900 °C is essential for synthesizing pure nanostructure forsterite. Cheng et al. [34] claimed that 30 h high-energy ball milling and subsequent calcination at 900 °C resulted in the formation of forsterite nanopowders. Mirhadi et al. [16] reported a combined sol–gel and ball milling (for 5 h) method followed by heat treatment at 750 °C for the fabrication of forsterite nanoparticles. Karbovnyk et al. [1] indicated that the forsterite-based ceramic nanopowder could be synthesized through long-time sol-gel assisted by further thermal treatment at 900 °C for 3 h. Taking the aforementioned facts into account, microwave irradiation technique possesses several significant advantages: 1) the whole process takes only a few minutes; 2) the obtained nano powder has high purity without any second phase; 3) the maximum temperature during the process is the boiling point of ethanol; 4) the cost is low; and 5) low calcination temperature is needed.
3.3. In vitro biological study

3.3.1. In vitro bioactivity evaluation

Bioactivity is one of the most important characters of a biomaterial since it causes a proper bonding between the biomaterial and surrounding bone tissue through a bone-like apatite layer that formed on the biomaterial surface. Fig. 6 shows SEM micrographs of the surfaces of forsterite samples after soaking in the SBF for different immersion times. After immersion for 14 days, spherical particles with cauliflower-like structure were precipitated on the surface of sample (Fig. 6a and b). It can be seen that after immersion for 21 days, cauliflower-like particles completely covered the surface of sample (Fig. 6c and d). After immersion for 28 days, the spherical particles were found to cover the whole surface by forming a continuous layer (Fig. 6e and f). EDS analysis of the surface of forsterite sample after soaking in SBF solution for 28 days showed that the precipitated particles were mainly composed of calcium and phosphorus elements. This is consistent with the formation of apatite layer on the surface of forsterite sample.

Fig. 7 shows concentration changes of Ca, Mg, Si, and P ions of the SBF solution after the soaking of the forsterite samples for different immersion times. It can be seen that the Ca and P ions concentration decreased with increasing the immersion time while there is an inverse trend for Mg and Si ions. The precipitation of bone-like apatite on the surface of samples is responsible for decreasing the Ca and P ions with increasing the immersion time. Conversely, Mg and Si ions concentration increased with increasing the immersion time, which may be attributed to the dissolution of forsterite samples.

The formation of a bone-like apatite layer on biomaterials surface is the essential requirement for them to bond to living bone. Indeed, the apatite-formation ability of a biomaterial is considered as the precondition for inducing bone. When a bioactive material is implanted in the human body, apatite would form on its surface due to the interaction between that material and physiological ions. This layer causes a biological fixation between bone tissue and the bioactive material [35–37]. Evaluation of in vitro bioactivity indicated that the synthesized forsterite nanopowder via microwave irradiation exhibited excellent apatite-forming ability in SBF; this indicates superior bioactivity.

3.3.2. Cell viability study

Fig. 8 shows the result of cell viability and cell proliferation of MG63 cells on the forsterite samples as well as TCP as control. It can be seen that there was no significant difference in cell proliferation between nanoforsterite samples and control group (TCP) at the first day. Moreover, the proliferation of MG63 cells cultured on the forsterite samples and TCP gradually enhanced from day 1 up to day 5 indicating no signs of cytotoxicity. The proliferation of MG63 cells cultured on the forsterite samples and TCP gradually enhanced from day 1 up to day 5 indicating normal cellular proliferation without any signs of toxicity. There was no significant difference in cell proliferation between forsterite samples and control group (TCP) at the first day. MTT assays after 3 and 5 days in culture not only showed a significant enhancement ($p < 0.05$) of cell proliferation on forsterite groups compared to the control group but also presented a significant improvement in viability.
trend with increasing time in culture that could be related to ions released from nanostructured forsterite. In other words, MTT assay results imply that nanoforsterite made by microwave-assisted method cause no inhibitory effect on cell viability and proliferation.

Similar trend was reported in previous studies. Xie et al. [38] considered cell behavior of mesenchymal stem cells on the titanium surface coated with nanoforstrite bioceramics. They reported gradually increase in cell proliferation through 6 days cell culture without any sign of cytotoxicity [38]. Scalera et al. [39] showed similar behavior in MG63 cells cultured on chitosan/nanoforsterite composite scaffolds. Zhai et al. [40] reported that this cell behavior is related to the stimulatory effects of the ionic products from silicate bioceramics.

Cell morphology study (Fig. 9) showed that the MG63 osteoblast like cells well attached on the forsterite sample and covered its surface. The cells were spread and started to form tissue layer in some areas of the sample. The shape of cells was mainly polygonal and in flatted form which approved MTT assay result and well cytocompatibility of microwave-assisted synthesized forsterite nanopowders.

The biological response to biomaterials is affected by several parameters such as their chemical composition, porosity, grain size, topography, and synthesis method. These parameters may change surface reactivity and consequently bone cell functions [41]. Several families of osteogenesis genes could be stimulated by ion dissolution products such as Si, Mg, etc. [42]. Several researches demonstrated that the presence of Si and Mg ions in the culture media stimulate osteogenesis genes expression, vascularization, and in vivo bone formation [40, 43]. As can be observed from Fig. 7, Mg and Si ion concentrations increased with increasing the immersion time because of dissolution of forsterite samples, which may affect MG63 osteoblast-like cell to more proliferation. The results indicated that forsterite nanopowder fabricated
method not only showed no cytotoxicity but also improved cell proliferation and cell morphology study indicated well-expressed phenotype of MG63 osteoblast-like cells. ALP activity experiments indicated that the fabricated forsterite nanopowder could facilitate the cells to proliferate and differentiate. Therefore, microwave-assisted synthesis technique could be considered as a novel, safe and high efficient method in saving time and energy for bioactive forsterite nano powder production.

Acknowledgements
Partial financial support by Center of Excellence for Surface Engineering and Corrosion Protection of Industries, University of Tehran, and Iran Nanotechnology Initiative Council are gratefully acknowledged.

References

Fig. 10. ALP activity of MG63 cells cultured on forsterite samples after 7 and 14 days of cell culture (* represents significant difference between the groups (p < 0.05)).