RESEARCH ARTICLE

Synthesis and Cell Seeding Assessment of Novel Biphasic Nano Powder in the CaO-MgO-SiO_2 System for Bone Implant Application

Kazem Marzban[1]* and Sayed Mahmood Rabiee[2]

[1]Department of Biomaterials, Science and Research Branch, Islamic Azad University, Yazd, Iran
[2]Department of Materials Engineering, Nanobiotechnology Research Group, Babol University of Technology, Babol, Iran

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ABSTRACT

Objective(s): CaO-MgO-SiO_2 system bioceramics possess good characteristics for hard tissue engineering applications. The aim of the study was to synthesize the nano powder by using a sol-gel method and evaluate of bioactivity in the cells culture.

Methods: To characterize of powder X-ray diffraction (XRD), transmission electron microscopy (TEM) and to evaluate the bioactivity sample cell seeding and methylthiazol tetrazolium (MTT) assay were performed.

Results: X-ray diffraction (XRD) analysis showed that the biphasic powder was obtained at 1300°C for 2 h by using a sol-gel method. Transmission electron microscopy (TEM) image showed that powder particle size was about 45 nm. Besides, cell culture results indicated that the percentage of viability values was increased by the extension of period.

Conclusions: found that the sample is cytocompatible and has cell proliferation potential in culture medium. The present study demonstrates that, the biphasic CaO-MgO-SiO_2 system can be used to achieve novel bioactive materials for bone implant application.

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INTRODUCTION

Bone tissue engineering possesses great potential for repairing bone defects of surgical resection, and congenital deformity [1]. Bioactive materials are qualified by their close connection with living bone via hydroxyapatite formation [2]. Newly, a variety of bioactive materials (e.g. biopolymers, bioceramics and their biocomposites) as bone implants are used. Ca-p ceramics are a type of bioactive materials that used in bone tissue regeneration [3]. However, for the reason of its poor chemical stability, cannot be preserved for long term stability [4]. The results indicated that the bioceramics base on Ca-Si-Mg might be used as novel bioactive materials for bone regeneration [5-8]. Ionic release of akermanite and merwinite considerably causes the adhesion and proliferation of cells [7-8]. The bioceramics not only have a good mechanical strength, but also having high chemical stability and biocompatibility [9]. As mention previously, some of silica-containing materials had shown higher bioactivity than calcium phosphate materials [10]. Adding magnesium to calcium and silicate compounds markedly enhance the chemical stability [11]. The results showed that the MgO content affected the mechanical properties and biological performances of bioceramics [5]. Osteoblasts adhesion, proliferation and differentiation

* Corresponding Author Email: *kazemmarzban@yahoo.com*

could be enhanced into the culture medium by Ca, Mg, Si ions dissolved and released [1]. Marzban prepared a $CaO-SiO_2$-MgO system ceramic and showed that it has apatite-formation ability in the simulated body fluid (SBF) [12]. Nakajima et al demonstrated the ceramics when implanted in rabbits could closely bond to the bone tissue. Found that the release of Ca, Mg, and Si from the surface have an important role in the cells adhesion and proliferation [13]. Ceramics such as Bredigite ($Ca_7MgSi_4O_{16}$), Diopside ($CaMgSi_2O_6$), Merwinite ($Ca_3MgSi_2O_8$), Akermanite ($Ca_2MgSi_2O_7$) and Monticellite ($CaMgSiO_4$) are in the system [7]. The elemental composition of merwinite and akermanite is almost the same (merwinite only has one more CaO than akermanite), But, the crystalline lattice of two bioceramics is different and have monoclinic structure and tetragonal structure, respectively [14]. Chen and et al proved that the MgO content has a significant impact on biological properties in the $MgO-CaO-SiO_2$ system. So that, the mechanical properties were increased from merwinite to akermanite with the increase of MgO contents, but the formation of bone-like apatite on the surface and cell proliferation decreased [15]. It seems akermanite doping into merwinite as a novel biphasic ceramics in $CaO-SiO_2$-MgO system might render better properties compared to the merwinite and akermanite alone. In other hand, a few researches have been done on bioactivity and cytocompatibility of a biphasic ceramics in $CaO-MgO-SiO_2$ system. Hence, it is necessary to investigate the biological characteristics of the ceramics as a successful candidate for bone implants. In this paper, we use the sol–gel method for biphasic nano powder production. The method has many advantages such as being performed at lower temperature, producing nano-sized particles, costing less and for high homogeneity [16].

METHODS

Preparation and Characterization Nano Powder

Nano powder was obtained by sol-gel method using tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS), magnesium nitrate hexahydrate ($Mg(NO_3)_2.6H_2O$) and calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) as the raw material of Si, Mg and Ca, respectively. Nitric acid (HNO_3) was used as a precipitant. TEOS was mixed with water and nitric acid (molar ratio: TEOS/H_2O/HNO_3=1:8:0.16) and stirred for 30 min. After that, the $Mg(NO_3)_2.6H_2O$ and $Ca(NO_3)_2.4H_2O$ was added into the solution (molar ratio: TEOS/$Mg(NO_3)_2.6H_2O$/$Ca(NO_3)_2.4H_2O$=2:1:3) and stirred at room temperature for 5 h and heated at 60 for 48 h. Finally, the gel obtained was heated at 1300°C for 2h. In the crystalline phases the powder was determined by X-ray Diffraction Analysis (XRD; Philips PW3710 diffract meter). Scanning Electron Microscopy (SEM, Philips XL30) and Transmission electron microscopy (TEM; EM208S) was used for microstructure observation. The crystalline size of nano powder was calculated according to Scherrer equation [15], as follow:

$$t = 0.89\lambda/\beta\cos\theta$$

Where t is the crystallite size, λ is the wave length, β is peak width chosen at half height in radians and θ is the Bragg angle.

Samples Preparation for Analysis

The powders were mounted stiffly on a specimen holder called specimen stub and coated with gold for 3 min by a sputter coater (Eiko IB3, Tokyo, Japan). The microscope to visualize the samples was adjusted at 25 kV. XRD at a scan rate of 0.02/min with Cu Kα radiation was used for the crystallographic structural analysis of the sample. Samples preparation has been done by obtaining a suspension from ultrasonification of the powder in ethanol on a foil surface followed by dropping on a cupper grid and finally dried to capture the images by means of TEM. Preparation of samples has been done by obtaining a suspension of the powder in ethanol, then, dropping on a cupper mesh and eventually dried to capture the Pictures by instruments of TEM.

Cell Seeding

The cytocompatibility evaluation of the ceramic disks was determined in culture medium with 5% CO_2 at 37°C. The Saos-2 cells were seeded on the samples for 24 h, 72 h and 168 h. Then, seeded disks were removed and fixed with 2.5% glutaradehyde buffer. After that, the disks were rinsed several times with phosphate-buffer solution (PBS). Finally were dehydrated sequentially in a grade ethanol series and the morphology of the cells on the disks was viewed by stereomicroscopy (Olympus IX71). The MTT test was used to assess the viability of the cells. Briefly, after the culture term (24 h, 72 h and 168 h), new culture and MTT solution were added

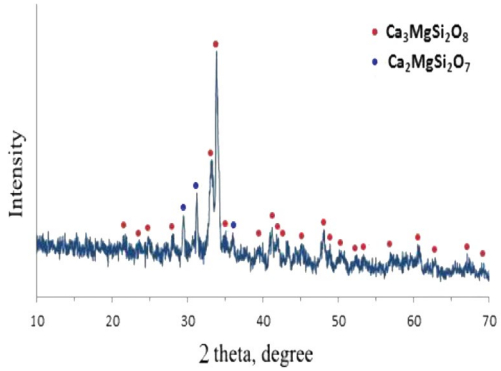

Fig. 1. XRD pattern of the powder sintered at 1350°C for 2 h.

to wells, 720 µL and 80 µL, respectively. The samples were maintained in the same culture conditions. Following that, the supernatant was removed. Plate culture (Ultra high molecular weight Poly Ethylene) was used for negative control. The cells viability percentage was measured by using spectrophotometry at a wavelength of 570 nm.

RESULTS AND DISCUSSION

The XRD pattern of powder obtained by sol-gel method after heat treatment at 1300°C for 2 h is shown (Fig. 1). The XRD peak position is in a good match with previous study (5). $Ca_3MgSi_2O_8$ (corresponding to JCPDS card no. 35-0591) and $Ca_2MgSi_2O_7$ (corresponding to JCPDS card no. 35-0592), as the main crystalline phase and the minor phase were identified, respectively. According to Scherrer equation [16], the particle size of the powder at this study is about 42 nm. The strong and sharp peaks in XRD pattern can be due to the good crystalliztion of powder.

The SEM and TEM micrographs of the structural morphology of the obtained powder are shown (Fig. 2). The micrographs of very small particles shows the irregular shape of the powder that stick together and that particles agglomerated as a result of high surface energy and being nano-sized powder [16]. The TEM micrographs of the powder confirm that, the particles size was about 45 nm.

The optical images of the morphological of Saos-2 osteoblast cells cultured on the disk ceramics for 72 h are shown (Fig. 3). According to light microscopic images of the samples after 72 h, a distinct cell distribution observed, which show high proliferation cell (white arrows). Furthermore, the stereomicroscopy images showed that (Fig. 3c) the cells seed was attached to disk edge and got confluent and elongated. Besides, proliferation of cells is clear around the disk (note that, the white line is the interface between culture medium and disk).

The results suggest that akermanite doping into merwinite is a useful approach to obtain bioceramis with biocompatibility properties, and might be promising candidates for bone tissue regeneration. Mihailova and et al prepared biphasic merwinite-

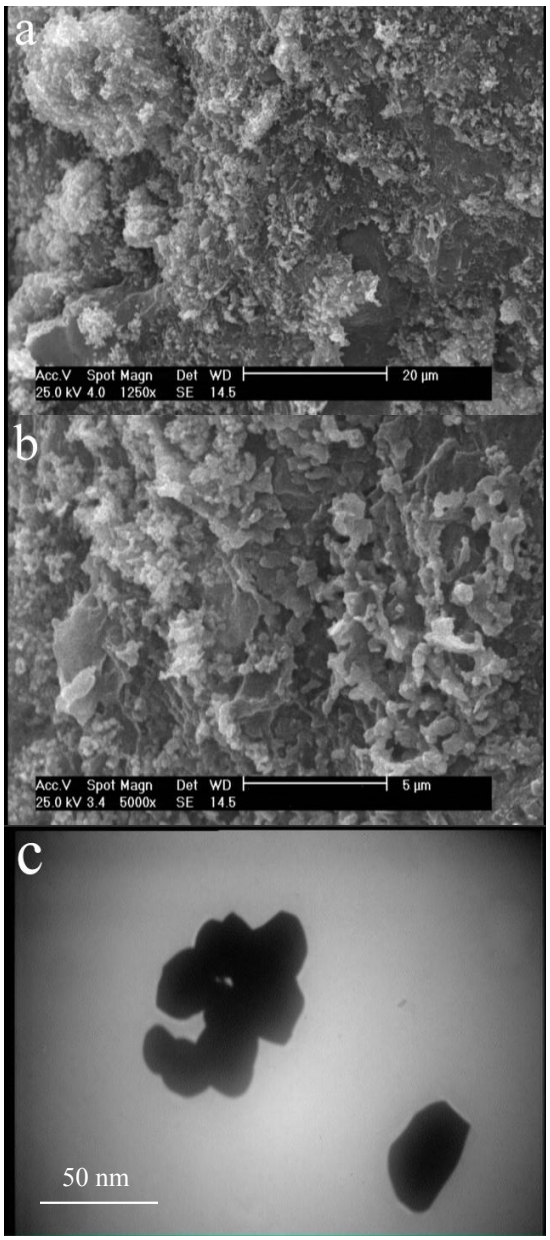

Fig. 2. (a, b) SEM and (c) TEM micrographs of the synthesize nano powder.

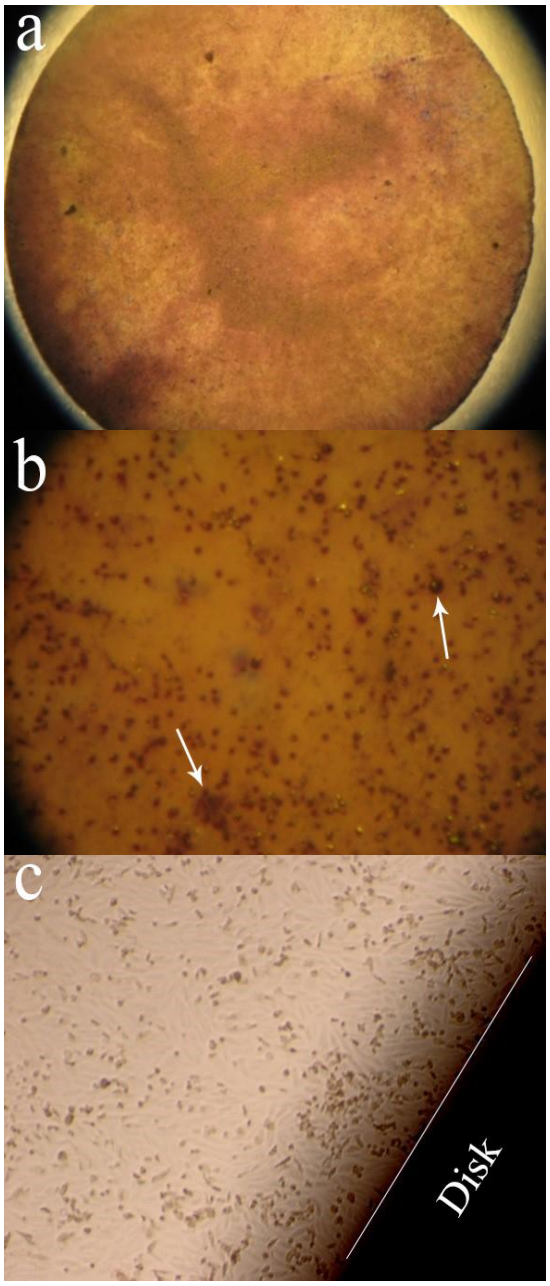

Fig. 3. (a) Shows optical microscopy (OM) images of Saos-2 osteoblast cells cultured on the disk for 72 h, (b) high magnification and (c) cells cultured in the interface between disk and culture medium

akermanite bioceramics, and then evaluated their structural properties and in vitro bioactivity. The results showed that the biphasic bioceramics had ability to form an hydroxyapatite (HA) layer in SBF solution (5). Also, the previous studies showed that calcium silicate with Mg bioceramics such as akermanite bioceramics have the high ability to stimulate angiogenesis, which cusses to bone repairing [17]. Radev and et al have been prepared a new biphasic calcium silicate ceramic with phosphate through polystep sol-gel method [18]. In another study, they prepared biphasic ceramic of akermanite and hydroxyapatite [19]. In both study after sample preparation, evaluated the bioactivity in static conditions in 1.5SBF. Their results indicated that apatite phase is covered on both surfaces after immersion. In general, the comparative studies are required to proper understanding the superiority of biphasic bioceramics properties in comparison with its single phase.

Generally, the MTT assay is used for measuring cellular viability. (Fig. 4) shows the MTT assay after different period of cell culture using Saos-2 osteoblast. It can be seen that the percentage of viability values of osteoblasts are significantly enhanced after being incubated for 24 h, 72 h and 168 h. The results of MTT assay showed that the percentage of viability values osteoblasts cell increased with the extension of culture period and this indicated the higher cell proliferation at 168 h. The Saos-2 osteoblast cultured showed a significantly proliferation rate at 24 h, 72 h, and 168 h. Fiocco and co-works studied porous glass-ceramic foams, with both wollastonite ($CaSiO_3$) and diopside as crystal phases. Similar to the results of the present paper, they observed the an increase in cell viability passing from 3 to 7 days for both the materials, indicates that the cells surviving at Day 3 might proliferated after 7 days [20]. Yi and et al prepared akermanite bioactive coatings through a plasma spraying method and its Biocompatibility evaluated in compared with apatite coatings. Proliferation rate on the two coatings shows no significant difference after 1 day of culture. Proliferation rate on the two coatings showed that when the culture time was increased from 1 day to 3 days and then to 7 days, cells proliferation rate on the akermanite coatings higher than that on HA coatings [6]. Also, Wu and colleagues prepared and characterized bredigite bioactive ceramic. The results showed that cell number increased significantly with the increase of culture time after 5 days of culture [21].

CONCLUSIONS

Biphasic bioceramics in CaO-MgO-SiO_2 system could be obtained by heat treatment at 1300°C for 2 h. It was found that the powder obtained at the study was about 45 nm. The results showed that the

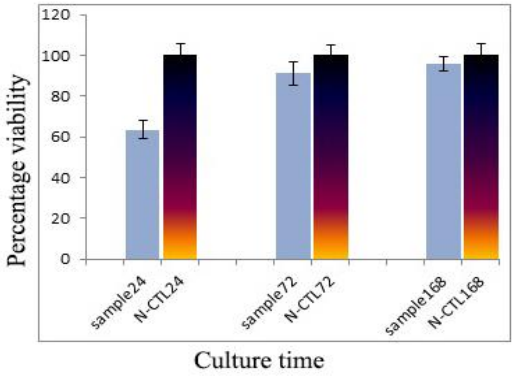

Fig. 4. Shows the MTT assay of the bioceramic after different times of cells cultured.

sample is cytocompatible and has cell proliferation potential in culture medium. The present study demonstrates that, the biphasic CaO-MgO-SiO_2 system can be used to achieve novel bioactive materials as bone implant. The novel biphasic bioceramics could be applied to bone defects in load-bearing areas, and provide appropriate link with new bone. The results indicated that ceramics containing these elements might be used as implant for bone regeneration.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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