Synthesis and Characterization of Hydroxyapatite/Bioactive Glass Nanocomposite Foam and Fluorapatite/Bioactive Glass Nanocomposite Foam by Gel Casting Method as Cell Scaffold for Bone Tissue

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ABSTRACT

The aim of this study was to synthesize and characterize of hydroxyapatite/bioactive glass (HA/BG) nanocomposite foam and fluorapatite/bioactive glass (FA/BG) nanocomposite foam by gel casting method as cell scaffold for bone tissue engineering and evaluating their bioactivity using in vitro methods. Brunauer-Emmett-Teller (BET), X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Archimedes method and universal testing machine were used in order to evaluate specific surface area, phase composition, shape, size and interconnectivity of pores, size and shape of the constructed foam particles, porosity measurement and compressive strength of prepared nanocomposite foams, respectively. Mean particle size of HA/BG and FA/BG nanocomposite foams were 78 and 42 nm respectively with average pore size ranges was from 100 to 400 µm for two compositions. The maximum values of compressive strength and elastic modulus of nanocomposite foams were 0.22 and 17.8 Mpa for HA/BG and 0.13 and 22 MPa for FA/BG, respectively. The mean values of the apparent and true porosity were calculated 31 and 78% for HA/BG and 42 and 77% for FA/BG, respectively. The results of in vitro immersion of foams in simulated body fluid (SBF) demonstrate the formation of apatite on the surface of foams that indicating their bioactivity. The prepared nanocomposite foams in this research are appropriate substitutes for the bone defects in tissue engineering due to their characteristics. Moreover, using gel casting method to introduce these bioceramics provides the possibility to construct foams by molding or 3D printing in a desired shapes in accordance to patient’s needs with high dimensional accuracy.

Keywords: nanocomposite foams, tissue engineering, cell scaffold, gel casting method, biomedical applications

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INTRODUCTION

Nowadays, biocompatible synthetic materials are used to replace the damaged tissues. However, due to little similarities between chemical, biological or physical properties of these materials and the host tissue, using them often fails and requires retreatment [1]. The limitations of conventional methods such as autograft, allograft and xenograft for this purpose include low availability, lack of sufficient mechanical strength and configurability, causing and transmission of disease in the recipient. Considering the critical arguments regarding to use of natural bone grafts, the development of synthetic materials made of metals, ceramics, polymers and composites for bone substitution is highly crucial [2].

The main feature of metal alloys is their appropriate mechanical property. However, there are always concerns about their bioactivity and ability to join with living tissue without external forces and resistance to their corrosion in physiological environment [3]. Contrary to metal alloys, there are known biocompatible ceramics and glasses with excellent biological properties that establish a strong chemical bond with tissue in a short time [4] and show appropriate biological response in the junction of bone and tissue and thus have wide applications in medicine [5, 6]. However, due to their inappropriate physical properties, their application is faced with problems in regions under mechanical load [3].

The main content of the mineral composition of natural bone consists of hydroxyapatite \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) nanocrystal structure (50 nm long and 5 nm wide). Several studies demonstrated that calcium phosphates and their derivatives such as hydroxyapatite can present a desirable environment for bone tissue regeneration due to their similarity to the chemical composition of natural bone, tooth and enamel [7].

These bioactive materials are also increasingly used in dentistry. Several studies have reported the positive effect of bioactive bioceramics on bone cells growth [3, 4]. The ideal biomaterials for orthopedics should support the mechanical properties and biocompatibility during the period of implantation [8]. The problems that limit the application of hydroxyapatite include high decomposition rate in biological systems and in vivo solubility which led to less use of hydroxyapatite in long-term applications [9]. However, its biological and physico-chemical properties can be reinforced by substituting ions that are normally present in natural bone apatite [10]. The intrinsic fragility of glass is the main limitation of using bioactive glass as tissue-engineering scaffolds [11].

It was found that insertion of fluoride ions into hydroxyapatite structure increases its resistance to biodegradability considerably. In addition, by better absorption of protein, expresses a stronger cellular connection and reinforces the activity of phosphates that make stronger osteoconductivity [12, 13]. If OH- groups in HA are completely replaced with F-, flourapatite \( \text{Ca}_{10}(\text{PO}_4)_6\text{F}_2 \) is formed [12], which has more chemical and structural stability compared with hydroxyapatite [14]. This ion substitution has positive effect on proliferation, morphology and differentiation of osteoblast-like cells and enhances bioactivity [12]. There is 1 wt. % fluorine (10,000 parts per million) in the cortical bone. The presence of this level of fluorine in the bone prevents loss of bone density, which is the cause of osteoporosis [15]. Flourapatite also forms the outer layer of tooth [16]. The mineral phase of tooth under the enamel contains about 0.04-0.07 wt. % fluorine [17]. The enamel consists of flourapatite with substitution of 50% F- with OH- [18].

The combination of bioactive glass particles with hydroxyapatite gives it special properties such as augmentation of bioactivity and mechanical properties [19]. Adding silicate ions into hydroxyapatite structure increases in vivo bioactivity and induces osteogenesis and angiogenesis during bone regeneration [20].

Reinforcing the properties of bioactive ceramics is possible through controlling important parameters such as size, distribution and density of particles. Considering the theoretical principles of the bioactivity mechanism of these materials, when the size of particles decreases, specific surface area that is exposed to interstitial fluid and cells increases. In other words, comparing two identical compounds with similar weight value, the sample with smaller particles has more bioactivity (release of ions that affect osteogenesis) [3, 5, 21]. As a result, their osteoconductivity, biodegradability, sintering capabilities and mechanical reliability can be reinforced by controlling the size of particles and its structural morphology down to the nanoscale [22].

The new challenge in biomaterials and tissue engineering is increasing self-regeneration capacity of the body by stimulating of genes at the lesion or damage site. The new generation (third) of bioactive glass and foams with high macroporosity, creates a framework for penetration and migration of cells through scaffold. Using these structures act as a pattern for bone growth in the three dimensions and stimulate tissue regeneration by activating genes [23-25]. The porous size plays a key role in design of scaffold and directly affects bone regeneration. The porosity allows angiogenesis with a size of 5 µm. Size of 15-40 µm allows the growth of fibroblast, 40-100 µm is appropriate for osteoid growth, 200-350 µm allows significant growth of bone and higher than 500 µm allows quick angiogenesis. According to different studies, the optimal size for osteoconductivity is 150-600 µm [25]. The mechanical properties of scaffold should provide sufficient mechanical stability before the regeneration of new tissue in areas under load [11].
Nowadays, there are various technologies for manufacturing high porosity bioceramics [11]. The gelcasting method is an appropriate method for manufacturing ceramic materials with high rigidity and dimensional accuracy, homogeneous structure and various complex forms [26-28]. In this process, foaming is done by adding foaming agents and vigorous agitation of aqueous ceramic suspension. Natural proteins and polysaccharides such as agarose are often used in new gelcasting systems [11, 29, 30].

The novelty of this study is combination of fluorapatite with bioactive glass in order to fabricate foam and compare its properties with hydroxyapatite/bioactive glass. The present study aims to synthesize and characterize hydroxyapatite/bioactive glass nanocomposite foam and fluorapatite/bioactive glass nanocomposite foam by gelcasting method as cell scaffold for bone tissue and evaluating their bioactivity using in vitro method.

MATERIALS AND METHODS

Preparation of HA/BG and FA/BG Nanocomposite Foams

Starting materials

Nanopowders of HA [Ca_{10}(PO_{4})_{6}(OH)_{2}], FA [Ca_{10}(PO_{4})_{6}F_{2}], and BG (58S), with a composition of 58% SiO₂, 36% CaO, and 6% P₂O₅ and particle size of <100 nm (Nikceram Razi, Isfahan, Iran), were developed via the sol-gel method and used as prefabricated composite foams. In addition, agarose powder (Merck, Darmstadt, Germany), Tergitol® NP-9 (Sigma-Aldrich, St. Louis, MO, USA), and tripolyphosphate (TPP; Sigma-Aldrich) were used in the gelcasting method.

Preparation of composite foam by gel casting method

1 wt. % TPP solution was prepared in deionized water as dispersing agent. A dispersing agent is necessary to form a slurry with high fluidity and with maximum loading of solid material, increasing the stability of foam. HA and BG nanopowders (at an equal weight ratio) were ground, mixed, added 60 wt. % to 1% TPP in deionized water, and mixed for 15 min. In addition, the 7 wt. % agarose solution was added to the mixture and mixed at 130°C to obtain a suspension containing 50 wt. % HA/BG and 1.2 wt. % active gelling agent. Finally, 3 vol. % Tergitol® was added to suspension as surfactant. The process of foaming was done through stimulation by a three-blade mixer at 80 °C for 15 min. The foamed product was poured into polyethylene molds with the intended shapes and dimensions and the gelation process was done through cooling the foam to 0 °C. Then, the samples were extracted from the molds and dried at room ambient and sintered at 1200 °C for 4 hours in a muffle furnace. For the synthesis of FA/BG nanocomposite foams, FA nanopowder was used instead of HA nanopowder.

In order to do analyses that require the powder form of composites, the foams were crushed and grinded with a manual mortar.

Characterization of Materials

Specific surface area

The specific surface area of prepared composite foams were measured by the N₂ adsorption isotherms using Brunauer-Emmett-Teller (BET) technique (Belsorp mini II, Bel, Japan).

X-ray diffraction analysis

X-ray diffraction (XRD) was carried out using X’Pert PRO MPD (PANalytical, Netherlands) to analyze the phase composition of the constructed composite foams with CuKα radiation with a wavelength of 0.1542 nm at 40 kV and 40 mA, with a step size of 0.02 degrees and a count rate one step per second. The diffraction patterns were recorded from 20° to 70° and were compared with the standards proposed by the Joint Committee on Powder Diffraction Standards (JCPDS). Finally results were analyzed by X’Pert High Score Plus (V. 3).

Electron microscopy analysis

Scanning Electron Microscopy (SEM, TeScan – Mira III, Czech Republic) was used to analyze the shape and size of pores and the morphology of composite foams. Image analysis method was used to determine the pore size distribution. The diameter of at least 50 pores was measured and the pore size distribution was calculated.

Transmission Electron Microscopy (TEM, Zeiss EM900, Germany) was used to study the size of the constructed foams particles.
Porosity measurement

In order to determine the porosity of the prepared composite foams, three samples of each composite foam were chosen and their porosity was measured by Archimedes’ method. The apparent porosity, which shows interconnected porosity, was determined by weighing the dry ceramic \( W_d \) and reweighing again while immersed in water \( W_w \) and after it is removed from the water \( W_s \) based on the following equation:

\[
\% \text{Apparent porosity} = \left( \frac{W_w - W_d}{W_w - W_s} \right) \times 100
\]

The true porosity includes the sum of interconnected and closed porosity, which has better correlates with ceramic properties and is calculated by the following equation:

\[
\% \text{True porosity} = \left( \frac{\rho - B}{\rho} \right) \times 100
\]

where

\[
B = \frac{W_d}{W_w - W_s}
\]

B is the prepared foam bulk density and \( \rho \) is the true density or specific gravity of the composite. The bulk density is achieved by dividing its weight by its volume [2].

Mechanical testing

To determine the mechanical properties of composite foams, compressive strength test is an appropriate criterion. To measure the compressive strength of the prepared porous foams, cylindrical samples with a height of 20 mm and cross-sectional diameter of 10 mm were tested by universal testing machine (SANTAM, Teharn, Iran) using 10 kN load cell and the speed of 0.5 mm per minute. The load was continued until the first crack appeared on the sample. Compressive strength was estimated from dividing the maximum load registered during the test by the main surface area. The elastic modulus was calculated according to the slope of the initial linear portion of the stress-strain graphs. The final results were presented based on the average results of the 3 samples of each type of composite [11].

In Vitro Assessment of the Bioactivity of Composites

To assess the bioactivity of the composites, a standard simulated body fluid (SBF) was prepared according to Kokubo’s protocol by dissolving appropriate amounts of chemicals including NaCl, NaHCO3, KCl, K2HPO4,3H2O, MgCl2.6H2O, CaCl2.2H2O, Na2SO4, Tris-hydroxymethyl aminomethane and CH2OH3-CNH2 in distilled water and was buffered with 2 molar hydrochloric acid to pH 7.25 at 36.5 °C. After sterilization, each sample was placed in a sterile polyethylene bottle and SBF was added to these bottles to achieve solid to liquid ratio 10 mg/mL. The bottles were kept in water bath (36.5±0.5 °C) for 3, 7, 14 and 28 days without changing SBF. At the end of each periods, one sample of each nanocomposites were rinsed with deionized water and dried at room temperature [31].

During this period, the pH of each sample solution was daily measured by digital pH meter (HANNA, HI-8424, Italy). The concentration of calcium and phosphorus of filtered solution was determined at the end of each period using Inductive Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Varian, 730-ES, USA). To confirm the formation of carbonate apatite layer on samples and study of functional groups, Fourier Transform Infrared (FTIR, PerkinElmer, RX I, USA) was used in the range of 400 to 4000 cm⁻¹. Moreover, formation of bone-like apatite on the surface of samples and pores filling due to apatite formation was examined by Scanning Electron Microscopy.

RESULTS

The specific surface area of nanocomposites obtained from BET were 0.40726 and 0.79761 g/m² for HA/BG and FA/BG nanocomposites, respectively with mean particle size of 78 and 42 nm. Figure 1 shows the TEM micrographs of prepared foams.
The X-ray diffraction patterns are shown in Figure 2. As the pattern shows, the more intense peaks at angles of 25.8, 32 degrees in HA/BG and 31.7, 32.9 degrees in FA/BG and mild peaks at angles of 46.8, 49.5 degrees in HA/BG and 25.8, 32.1 degrees in FA/BG, indicate the presence of apatite according to JCPDS9-432 standard. The peaks were observed in the angles of 27, 32 and 46 degrees and 22 degrees in both composites are related to wollastonite (CaSiO3) and silicon oxide (SiO2), respectively.

SEM micrographs of prepared nanocomposite foams shows the shape, size and connectivity of pores (Figure 3). As illustrated in the figure, the average pore size ranges from 100 to 400 µm. The apparent and true porosity calculated for the average results of 3 samples of each type of nanocomposite foam using Archimedes’ method were 31 and 78% for HA/BG and 42 and 77% for FA/BG, respectively.
As it could be seen in Figure 4, the range of compressive strength and elastic modulus in the nanocomposite foams was measured to be 0.1-0.22 and 9.6-17.8 MPa for HA/BG and 0.1-0.13 and 10.6-22 MPa for FA/BG, respectively.

Figure 5 shows the micrographs of prepared foams after immersion in SBF taken by SEM. As can be seen, bone-like apatite forms on the inner wall of pores in both composites and this leads to pore filling and transformation of pores.

FTIR analysis of immersed foams confirmed that a new apatite layer is formed on the foam surface after immersion in SBF and the particles observed in SEM are carbonate hydroxyapatite (natural apatite) that related to the peak associated with vibrations of carbonate groups (1412-1420 cm⁻¹: C-O asymmetric stretching v₃).
Figure 5. SEM micrographs of HA/BG and FA/BG nanocomposite foams after 3 days of immersion in SBF at 3000X magnification.

Figure 6. FTIR spectra of HA/BG and FA/BG nanocomposite foams before and after 3 and 28 days of immersion in SBF.
Figure 7. pH trends and dissolution profile graphs of SBF after 3, 7, 14 and 28 days immersing of HA/BG and FA/BG nanocomposite foams

Table 1. Maximum compressive strength of HA/BG and FA/BG nanocomposite foams before and after 3, 7, 14 and 28 days immersion in SBF

<table>
<thead>
<tr>
<th>Soaking Time(days)</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA/BG Compressive strength(MPa)</td>
<td>0.22</td>
<td>0.15</td>
<td>0.05</td>
<td>0.07</td>
<td>0.75</td>
</tr>
<tr>
<td>FA/BG Compressive strength(MPa)</td>
<td>0.13</td>
<td>0.06</td>
<td>0.04</td>
<td>0.025</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Figure 7 shows the diagrams of daily pH changes and the dissolution profile of prepared foams at the end of immersion in SBF. The diagram of pH changes has an initial linear slope until the 4th day and stays almost constant after that. Considering the calcium release profile, we can observe that the concentration of calcium increases as the immersion time increases and concentration of phosphorus decreases.

The maximum tolerated compressive strength of foams before and after immersion in SBF is presented in Table 1.
DISCUSSION

The main purpose of using nanopowders and gelcasting method in order to make nanocomposites, was to create more specific surface area. According to TEM micrographs, mean particle size of nanocomposite foams was in nanoscale and less than 100 nm. Decrease of particle size due to increase contact surface with body fluids increases osteoblast function including proliferation, alkaline phosphatase synthesis and sequestration of minerals containing calcium [32-34]. Achieving such a property is effective for increasing the bioactivity and the effectiveness of biomaterials to be used as cell scaffold in bone tissue engineering and provides fast tissue regeneration conditions. The porous bioceramics have been used to fill bone defects, stabilization of implant through internal growth of bone, bone regeneration through tissue engineering [35-37], drug delivery [38,39] and cell loading [40, 41]. High specific surface area of porous bioceramics has led to excellent osteoconductivity and increases cell adhesion to the scaffold and promotes bone regeneration [2, 35].

X-ray diffraction patterns in addition to show similar behavior of both prepared composites to sintering temperature, displays the peaks of apatite phase, amorphous phase, different amounts of tricalcium phosphate Ca₃(PO₄)₂ and calcium silicate CaSiO₃ (Figure 2). Since the BG is amorphous, it does not have a peak in X-ray diffraction and its combination with apatite decreases the intensity of apatites peaks [7]. The effect of sintering temperature on BG is creating two phases of wollastonite (CaSiO₃) and silicon oxide (SiO₂) that their peaks were observed respectively in the angles of 27, 32 and 46 degrees and 22 degrees in both composites, which is consistent with the results of other studies [2, 11]. It should be noted that sintering at 1200 °C increases the crystallinity of BG. BG crystallization affects its bioactivity and bioabsorption [42]. Moreover, this temperature converts a part of HA to TCP, which was observable in angles of 30 and 34 degrees in XRD pattern and was introduced by other researchers [2, 11]. Because of calcium phosphate phase is bioactive and biodegradable, its presence is not unfavorable but has useful effects on drug delivery processes and bone tissue engineering [2]. Since the solubility of TCP is more than apatite, its presence accelerates the biodegradability rate of the final composite [43].

Potoczek et al. constructed hydroxyapatite and calcium phosphate foams with pore size ranging 130-380 and 250-900 µm [44]. Hydroxyapatite foams constructed by Ramay et al. had 200-400 µm pores [45]. Ghomi et al. achieved to make HA/BG nanocomposite foams with 100-250 µm pores [2]. Since the minimum porosity required for active bone growth with feeding from the blood and body fluids is 100-150 µm [46, 47] and considering that bigger pores considerably decrease foam stability [35], achieving minimum pore size with the ability to feed on the adjacent tissue is extremely important [2].

Results show that about half of the porosities are open and are exposed to the body’s physiological fluids and cells when they are used in the body, which is necessary for their bioactivity. The interstitial connection between pores allows circulation and exchange of body fluids, ion diffusion, food supply, osteoblast cell infiltration and angiogenesis [35, 48].

Figure 4 shows compressive stress/strain graphs of each composite. As can be seen, the first part of the graph is almost linear and the slope of this part is equivalent to elastic modulus. The turning point of the graph is equivalent to maximum compressive strength the composite foam can tolerate and the first crack on the surface of sample occurs in this part. However, since the foams are porous, the negative slope changes to positive after delamination occurs in one layer of the porosities due to foam resistance and this process continues until all porosities are delaminated and accumulated. Ghomi et al. managed to make HA/BG nanocomposite foam with apparent and true porosity in the range of 86-91% and 60-71%, respectively, compressive strength of 0.87-1.95 MPa and elastic modulus of 92-204 Mpa [2]. The compressive strength of hydroxyapatite foams constructed by Potoczkek et al. with a porosity of 73-92% was equal to 0.8-5.9 Mpa [49] and calcium phosphate foams with a porosity of 90% was equal to 0.44-1.92 Mpa [44]. Sepulveda et al. created HA foams with a porosity of 72-90% and compressive strength of 1.5-5.8 Mpa [50]. The compressive strength is expected to be increased as the porosity decreases. According to our analysis, contrariwise above, the compressive strength decrease as the porosity decreases. Anyway, the foams need to have the minimum compressive strength of the trabecular bone to be used as appropriate substitutes for bone tissue [51]. Misch et al. calculated the compressive strength of mandibular trabecular bone 0.22-10.44 Mpa [52]. For this reason, manufactured foams in this study can be appropriate for bone defects which are not under load such as dentistry, however, they are not advised for parts of the body that must endure a lot of load.

The results of in vitro immersion of foams in simulated body fluid demonstrate the formation of apatite on the surface in exposure to SBF, indicating its bioactivity.

In order to ensure the formation of apatite and determine the functional groups, samples were taken from foams surface, before and after immersion in SBF, and were analyzed using FTIR analysis (Figure 6). Since the both HA/BG and FA/BG composites initially contain apatite compound, observing peaks associated with vibrations of phosphate groups (430-470 cm⁻¹: O-P-O bending v₁; 560-600 cm⁻¹: O-P-O bending v₄; 935-985 cm⁻¹: P-O stretching v₃; 1040-1070 cm⁻¹: P-O stretching v₂) in the spectrums before and after immersion in SBF is normal [11, 53, 54].
However, the peaks of $v_1$ vibration in the wavelength of 935-985 cm$^{-1}$ in both composites were excluded after immersion in SBF. But, the compound that differentiates the newly made bone-like apatite (carbonate hydroxyapatite: natural form of hydroxyapatite in bone), from the initial apatite synthesis in the combination of foams is the peak associated with vibrations of carbonate groups (1412-1420 cm$^{-1}$: C-O asymmetric stretching $v_3$), which is clearly observed in the spectrum of foams immersed in SBF [3, 4, 48]. 2357 cm$^{-1}$ and 1978-1998 cm$^{-1}$ peaks are associated with C=O and C-H bonds, respectively, which can be observed in samples before and after immersion in SBF and are the result of sintering at 1200 $^\circ$C and absorbing carbon from the environment. 735-755 cm$^{-1}$ and 3700-3800 cm$^{-1}$ peaks are associated with hydroxyl groups [12, 53, 54]. The peak observed in the range of 3420-3430cm$^{-1}$ is associated with vibrations of absorbed water bond [7]. Results of FTIR analysis demonstrated that strong bonds of bioactive silicate glass group (795-815cm$^{-1}$: Si-O symmetric stretching $v_3$) are observed in the foams with both composites, in addition to phosphate and carbonate groups of bone-like apatite [55-58].

In pH diagram (Figure 7), the pH changes in SBF solution without the immersion of foam is used as control. According to diagrams of daily pH changes and the dissolution profile, it is concluded that pH changes follow foam dissolution and pH increases with dissolution, which is consistent with the study of Ghomi et al. [11].

The primary SBF has 63.6 ppm calcium and 29.45 ppm phosphorus. Considering the calcium release profile, we can observe that the concentration of calcium increases as the immersion time increases because the speed of calcium release is higher than its decomposition on the surface of the foam, indicating the bioactivity of the foams. On the other hand, as immersion time increases the concentration of phosphorus decreases due to formation of hydroxy carbonate apatite layer on the surface of foams and slow dissolution of phosphorus from foam in SBF. These results have been reported by other researchers [11, 59-61]. It is important to note that in addition to proving the bioactivity of both composites and lack of significant difference between them in this test, it is observed that their main biological activities occur with steep slope during the early days of immersion in SBF and then progress slowly.

Considering the higher degradation rate of apatite than condensation of natural carbonate apatite after immersion in SBF, it is expected that the compressive strength of foams decrease as the remaining inside the body or SBF. According to Table 1, it can concluded that maximum changes in tolerated compressive strength of immersed nanocomposite foams in SBF, are appear in early few days, which is in agreement with results of calcium and phosphorus concentration and pH changes diagrams.

**CONCLUSION**

The prepared nanocomposite foams in this research are appropriate substitutes for the bone defects in tissue engineering due to their characteristics such as particle size, porosity and pore size, interstitial connection between pores, compressive strength and bioactivity. Moreover, using gelcasting method for these bioceramics provides the possibility to construct foams by molding or 3D printing in a desired shapes that is in accordance with the patient’s needs and has high dimensional accuracy.

Both HA/BG and FA/BG bioceramics constructed in this study contained equal proportions of apatite and bioactive glass and it was found that we can achieve optimum physical and biological properties by changing these proportions, the amount of polymer materials used and changing the sintering temperature. Further studies to analyze the biological properties such as antimicrobial activity, cytotoxicity, genotoxicity, etc. in accordance with international standards of biomaterials usable in human, in vivo assessments in animal models, and a comparison between them to determine the advantages of each one are seem be necessary.

**CONFLICT OF INTERESTS**

The authors declare that there is no conflict of interests regarding the publication of this paper.

**ACKNOWLEDGEMENT**

The authors gratefully acknowledge the financial support for this work by Babol University of Medical Sciences. This was an approved research proposal in Babol University of Medical Sciences # 9441219.

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