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Physicochemical properties and bioactivity of freeze-cast chitosan nanocomposite scaffolds reinforced with bioactive glass

Masoud Pourhaghgouy [a], Ali Zamanian [a,*], Mostafa Shahrezaee [b], Milad Pourbaghi Masouleh [a]

[a] Department of Nanotechnology & Advanced Materials, Materials & Energy Research Center, Karaj, P.O. Box: 13145-1659, Iran
[b] Department of Orthopedic Surgery, AJA University of Medical Sciences, Tehran, Iran

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ABSTRACT

Chitosan based nanocomposite scaffolds were prepared by freeze casting method through blending constant chitosan concentration with different portions of synthesized bioactive glass nanoparticles (BGNPs). Transmission Electron Microscopy (TEM) image showed that the particles size of bioactive glass ($64SiO_2.28CaO.8P_2O_5$) prepared by sol–gel method was approximately less than 20 nm. Fourier Transform Infrared Spectroscopy (FT-IR) and X-ray Diffraction (XRD) analysis showed proper interfacial bonding between BGNPs and chitosan polymers. Scanning Electron Microscopy (SEM) images depicted a unidirectional structure with homogenous distribution of BGNPs among chitosan matrix associated with the absence of pure chitosan scaffold's wall pores after addition of only 10 wt.% BGNPs. As the BGNP content increased from 0 to 50 wt.%, the compressive strength and compressive module values increased from 0.034 to 0.419 MPa and 0.41 to 10.77 MPa, respectively. Biodegradation study showed that increase in BGNP content leads to growth of weight loss amount. The *in vitro* biomineralization studies confirmed the bioactive nature of all nanocomposites. Amount of 30 wt.% BGNPs represented the best concentration for absorption capacity and bioactivity behaviors.

1. Introduction

Bone tissue engineering is a developing field, whereby cells are allowed to proliferate and organize their extracellular matrix in a three-dimensional lattice to form a functional tissue, exhibiting properties identical to native, healthy tissue [1]. Structural geometry of scaffolds is one of the important characteristics which provides an appropriate environment for cell attachment, growth and finally formation of new bone tissue [2]. Freeze casting or ice templating method is one of the promising ways to produce interconnected porous materials containing unidirectional channels as a result of unidirectional freezing of liquid suspension (aqueous or non-aqueous) which have some special advantages like controlled pore size distribution, environmentally friendliness, slight processing contraction and high mechanical strength [3–5]. However, some scaffold's material properties still need to be studied to fulfill the requirements of bioactivity, degradability and adequate biological responses in order to be applied in hard tissues [6,7]. Based on the fact that bone is a naturally mineralized composite, it is obvious that one biomaterial type does not possess all the mechanical/chemical properties that are necessary for such applications [8]. Flexibility of some polymers which facilitate implantation process is an advantage over more rigid materials with inherent brittleness and low toughness features that are unsuitable for load-bearing applications. Hence, biocomposites based on flexible biodegradable polymers and inorganic elements like bioactive glasses or bio ceramics, which exhibit the required mechanical properties and bioactivity, compared with polymeric scaffolds alone, have been developed for applications in bone repair and reconstruction [9]. Chitosan which is produced by deacetylation of chitin is a linear polysaccharide, based on glucosamine units, that can be found in sub-product of shellfish such as crabs and shrimps [10]. It is considered as a suitable functional polymer for biomedical applications due to its good biocompatibility, biodegradability, anti-inflammatory effect, protein adsorption properties and ability of accelerating wound healing. Its degradation products are non-toxic, non-antigenic, non-immunogenic and non-carcinogenic [11–13]. Furthermore, the positive surface charge of this biomaterial and its structural similarities with glycosaminoglycans, a major component of bone and cartilage, enable it to effectively enhance cell adhesion, proliferation, and differentiation [14,15]. However, chitosan by itself is not an ideal material for bone regeneration; its osteoconductivity and bio mineralization capability need to be improved. In general no apatite can precipitate on the surface of chitosan scaffold [16]. The addition of appropriate inorganic components such as bioactive glass to chitosan matrix leads to inducing biomineralization capability to prepare composite. This ability results in formation of apatite layer on bioactive glass which allows creation of safe chemical bonding between

* Corresponding author.
E-mail addresses: m.pourhaghgouy@merc.ac.ir (M. Pourhaghgouy), a-zamanian@merc.ac.ir (A. Zamanian), moshahrezaee@yahoo.com (M. Shahrezaee), miladpourbaghi@gmail.com (M.P. Masouleh).

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composite and living bone [17–19]. Since bioactive glasses are of surface reactive biomaterials, physical properties such as particle size, morphology and surface area can facilitate bone healing. Therefore, their addition in nanoscale range is more preferred [20–23].

In this study, bioactive glass nanoparticles (BGNPs) were synthesized through sol–gel method to fabricate chitosan based nanocomposite scaffolds by freeze casting method. Pure chitosan scaffold also was prepared to investigate the effect of BGNP content on morphology, physicochemichal characteristics, absorption behavior and mechanical properties of chitosan scaffold. Furthermore, *in vitro* biodegradation and biomineralization studies were investigated to evaluate the potential bone integration ability of each porous nanocomposites.

2. Materials and method

2.1. Materials

TEOS: $C_8H_{20}O_4Si$, nitric acid: HNO_3, ethanol: C_2H_6O, TEP: $C_6H_{15}O_4P$, calcium nitrate tetrahydrate: $(Ca(NO_3)_2.4H_2O)$ and ammonia: NH_3 for synthesis of BGNPs and also acetic acid were purchased from Merck Company (Darmstadt, Germany). NaCl, KCl, $NaHCO_3$, $MgCl_2 \cdot 6H_2O$, KH_2PO_4, TRIS: $C_4H_{11}NO_3$, $CaCl_2$ and HCl for preparation of simulated body fluid (SBF) and also chitosan (MMW: 190–310 kDa, DD: 75–85%) were purchased from Sigma Aldrich (Darmstadt, Germany).

2.2. Synthesis of BGNPs based on SiO_2–CaO–P_2O_5

BGNPs with formula of SiO_2:CaO:$P_2O_5 \approx 64:28:8$ (mol) were synthesized through sol–gel method. Concisely, mixture of TEOS (43.67 g) and distilled water (174.67 ml) was poured into a mixture of nitric acid (5.5 ml, 2 mol/L) and ethanol (87.33 ml) and stirred for 30 min. 8.38 ml of TEP was added to solution and stirred for another 30 min. The solution was stirred for also another 30 min after addition of 20.16 g calcium nitrate tetrahydrate. After that, the solution was aged for 1 h on stirrer. Finally, ammonia solution (2 mol/L) was added drop wisely to the solution under vigorous stirring until conversion of solution to gel was observed. The obtained gel was dried in oven (at 70 °C) to eliminate the residual water and ethanol. Then, dried powders were placed in furnace, heat treated at 700 °C for 2 h with heating rate of 3 °C/min to remove organics in order to form of glass particles (stabilization process). After that the powders were cooled slowly down in the furnace [24].

2.3. Preparation of scaffolds

The pure chitosan and chitosan-BGNPs nanocomposite scaffolds were prepared by freeze casting method. Chitosan (3 g) was dissolved in deionized water containing 2% of acetic acid by volume, at 30 °C, while stirring for 8 h to produce a homogenous solution of 3 wt.% chitosan. Synthesized BGNPs with different percentages of 10, 30 and 50 wt.% (toward amount of chitosan) were homogenously suspended in chitosan solution using ultrasonicator and a magnetic stirrer instrument. After freeze casting process, porous chitosan and chitosan-BGNPs nanocomposites were obtained. Freeze casting of prepared solutions was performed by pouring the solutions into a polytetrafluoroethylene (PTFE) mold with an inner diameter of 20 mm located on a copper cold finger. While liquid nitrogen was poured into the container, the cooling rate was controlled by a ring heater and a thermocouple which both were connected to a proportional-integral-derivative (PID) controller. A schematic illustration of freeze casting setup and prepared scaffolds is shown in Fig. 1. The cooling rate applied in this study was 1 °C/min. After a very careful removal of frozen samples from the mold, samples were dried in the freeze dryer (FD-10, Pishtaz Engineering Co. Tehran, Iran) at temperature of −55 °C and the pressure of 2.1 Pa for 48 h, in order to sublime the ice crystals. All samples were neutralized with 0.1 N NaOH for 1 h. Then, the excess base agents was removed by repeated washing with deionized water until the pH returned to the physiological range [25] and then freeze dried again. Prepared samples were maintained in a silica gel desiccator for further characterizations.

2.4. Transmission electron microscopy (TEM) observation

The morphology of the BGNPs was observed by TEM instrument (GM200, PEG, Philips), operated at an accelerating voltage of 200 kV. For TEM analysis, the BGNPs were ultrasonically dispersed in ethanol for 15 min and then few drops were placed on the carbon-coated copper grids.

2.5. Porosity of composite

The porosity value of the nanocomposites with different BGNP contents was calculated by following formula:

$$\text{Porosity}(\%) = ([V_t - (W_{Chi}/\rho_{Chi}) - (W_{BG}/\rho_{BG})]/V_t) \times 100 \quad (1);$$

where V_t is the total volume of nanocomposite scaffold (cm^3), W_{Chi} and W_{BG} are the mass of chitosan and BGNPs in the scaffold (g), ρ_{Chi} and ρ_{BG} are the density of chitosan and synthesized BGNPs which were measured by automatic density analyzer (Micrometrics, AccuPyc 1330 Pycnometer, USA) with amount of 1.4499 ± 0.0083 (g/cm^3) and 1.35 ± 0.0034 (g/cm^3), respectively. Triplicate measurements were carried out for each nanocomposites.

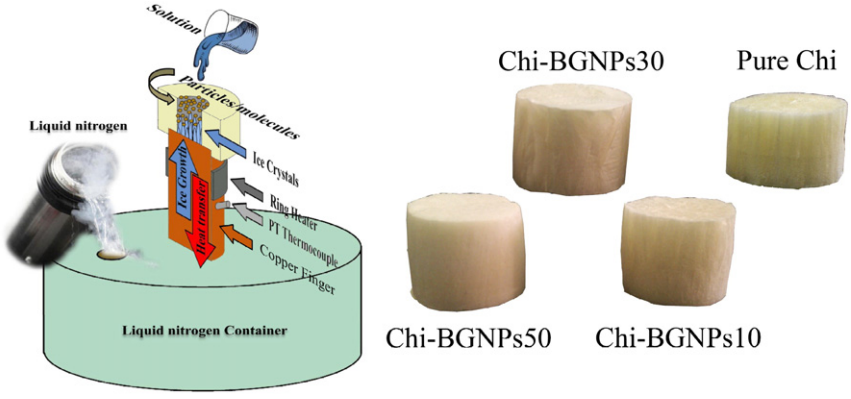

Fig. 1. Schematic illustration of freeze casting setup (Left side) and prepared scaffolds (right side). For example Chi-BGNPs10 code is indicative of 3 wt.% chitosan which contains 10 wt.% BGNP and freeze cast with cooling rate of 1 °C/min.