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Processing and in vitro bioactivity of high-strength 45S5 glass-ceramic scaffolds for bone regeneration

Ena A. Aguilar-Reyes[a,*], Carlos A. León-Patiño[a], Esmeralda Villicaña-Molina[a], Víctor I. Macías-Andrés[a], Louis-Philippe Lefebvre[b]

[a] Instituto de Investigación en Metalurgia y Materiales, Universidad Michoacana de San Nicolás de Hidalgo, Edif. U, Av. Francisco J. Múgica S/N Ciudad Universitaria, C.P. 58030 Morelia, Michoacán, México

[b] National Research Council Canada (NRC), Boucherville Research Facilities, 75 de Mortagne Boulevard, Building BOU-1, Boucherville, Quebec, Canada J4B 6Y4

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ABSTRACT

In this paper, the compressive strength and in vitro bioactivity of sintered 45S5 bioactive glass scaffolds produced by powder technology and polymer foaming were investigated. The sintering temperature of scaffolds was 975 °C. The characterization of scaffolds before immersion in SBF was performed by scanning electron microscopy (SEM) and microtomography (μCT). The scaffolds were also tested for compression, and their density and porosity were measured. After immersion, the samples were observed through SEM and analyzed using EDS, X-ray diffraction (XRD), and infrared spectroscopy (FT-IR). Mass variation was also estimated. The glass-ceramic scaffolds showed a 61.44 ± 3.13% interconnected porosity and an average compressive strength of 13.78 ± 2.43 MPa. They also showed the formation of a hydroxyapatite layer after seven days of immersion in SBF, demonstrating that partial crystallization during sintering did not suppress their bioactivity.

1. Introduction

The potential of bioceramic materials for tissue regeneration has been shown in vitro and in clinical practice. Certain bioactive glasses that have the ability to regenerate both soft and hard tissues are composed of these materials. The bioactivity of a material has been associated with the formation of hydroxyapatite crystals on the surface that is in contact with natural or simulated body fluid (SBF), similar to the inorganic structure of bone. In addition, bioactive glasses have been shown to exert control over the production of osteoblasts in the cell cycle [1]. This discovery has stimulated research on the use of bioactive glasses as scaffolds for tissue engineering. It has been demonstrated that bioactive glass 45S5, also known as Bioglass®, has the greatest potential to be used as a three-dimensional matrix (regenerative scaffold) in a large number of human bone components; although it crystallizes during sintering, its bioactivity slows down but it is not eliminated [2–4]. Recent studies have shown that the ability to regenerate human tissue through the formation of a hydroxyapatite surface layer depends on the porosity of the 3D bioactive glass structure, given that the scaffold has greater capacity when it is more porous [5–8]. Note that this porosity should be interconnected with proper pore size (300–500 μm) to enable cell infiltration, tissue ingrowth and vascularization, and nutrient delivery to the center of the regenerated tissue [7]. For these reasons, research continues to study the different ways of producing Bioglass® foams with characteristics similar to those of human bone. Currently, three methods are used to produce porous foams: the replica technique, the sacrificial template technique, and the direct foaming technique [9]. In the replica technique, a polymeric sponge (e.g. a polyurethane foam) is initially dip coated in a glass powder suspension, followed by oven drying and burning out of the polymer template. Finally, the glass or glass-ceramic structure is densified through sintering at high temperatures. The sacrificial template method involves the preparation of a composite made up of a sacrificial phase mixed with glass particles. The sacrificial phase is extracted (usually thermally) from the partially consolidated matrix to generate pores within the microstructure. This method leads to porous materials that display a negative replica of the original sacrificial template, as opposed to the positive morphology obtained from the replica technique described above. In the direct foaming method, a gas is incorporated into a glass or ceramic slurry to produce a foam, typically by bubbling air or an inert gas through the slurry.

The main objective of the present study was to implement a methodology to obtain bioactive scaffolds from Bioglass® powders and to examine the relationships between their microstructure and

* Corresponding author. Present/permanent address: Instituto de Investigación en Metalurgia y Materiales, Universidad Michoacana de San Nicolás de Hidalgo, Edif. U, Av. Francisco J. Múgica S/N Ciudad Universitaria, C.P. 58030 Morelia, Michoacán, México.
E-mail address: aareyes@umich.mx (E.A. Aguilar-Reyes).

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bioactivity. The combined method of powder technology and polymer foaming control the porosity, pore size, and compression strength of the scaffolds by varying the ratio of the foaming agent/binder/Bioglass® powder and the sintering temperature. This work was based on the principle that it is possible to obtain controlled re-absorption and dissolution rates of species that promote tissue regeneration through the production of glasses with a structure that mimics that of trabecular bone. The in vitro bioactivity of the Bioglass® scaffolds was monitored by evaluating the formation of the calcium phosphate layer on their surfaces after soaking in SBF.

2. Materials and methods

2.1. Preparation of the 45S5 bioactive glass

The 45S5 bioactive glass was obtained through the traditional melting-quenching technique of a mixture of high-purity SiO_2, Na_2CO_3, CaO, and P_2O_5 (Sigma-Aldrich, St. Louis, MO, USA) powders that were stoichiometrically prepared to produce the final composition of 24.5Na2O-24.5CaO-6P2O5-45SiO_2 (wt%). After being dry-mixed in a conventional ball mill for 30 min, the powder was placed in a fused silica crucible and heated to an intermediate step at 900 °C for 90 min to degasify the melt, followed by a second step at 1350 °C for 90 min in a Carbolite HTF 17/10 Furnace. The melt was then quenched in air on a stainless steel plate. Next, the glass was dry-ground in a Siebtechnik T750 Laboratory Disc Mill for 1 min and sieved to obtain fine powders with a particle size smaller than 63 μm (d_{10}=2.7 μm; d_{50}=21.9 μm and d_{90}=61 μm), as determined by the light scattering technique (Beckman Coulter LS 13 320; Beckman Coulter, Brea, CA, Fraunhofer optical model).

2.2. Fabrication of 45S5 Bioglass® glass scaffolds

The 45S5 bioactive glass scaffolds were produced through the combination of powder technology and the polymer foaming method [10]. The glass powder was dry-mixed with a solid polymeric binder (Varcum 29217, Durez Corporation, Niagara Falls, NY, USA) and a foaming agent (p-toluenesulfonyl hydrazide or TSH, Sigma-Aldrich, St. Louis, MO, USA) at a ratio of 45/54.5/0.5 in wt%, respectively. The mixing of the powders was performed for 45 min in a Shake-Mixer Glen Mills WAB, Model T-F2, using a glass container and 10-mm-diameter stainless steel balls. The powder mixture was then poured into an alumina mold (3 cm in diameter, 5 cm in height and 5 mm in thickness) for foaming. The foaming was carried out in a Thermolyne FB1300 Muffle Furnace. Afterwards, the foams were rectified into small cylinders of 10–18 mm in diameter and 20–30 mm in length, followed by the debinding and sintering steps, which were done in a Carbolite HTF 17/10 Furnace.

2.3. Bioactivity tests

The cylindrical scaffolds were cut into discs with dimensions of 10 mm in diameter and 3 mm in thickness. Prior to the bioactivity tests, the discs were sterilized in an ultrasonic bath with ethanol and acetone for 30 min. Next, they were dried in an oven for 24 h and exposed to ultraviolet irradiation for 40 min. Various times were selected for immersion in SBF: 1, 3, 7, 14, 21, and 28 days, using three samples for each immersion time. Each disc was immersed in 17.5 ml of acellular simulated body fluid (SBF), following the protocol published by Kokubo et al. [11]. The immersed discs were maintained at 37 °C in polyethylene vials under sterile conditions in a cell culture room. After soaking for different periods in the SBF, the specimen was taken out of the SBF and gently washed with pure water. The specimen was dried in an oven at 90 °C for 24 h and subsequently placed in a desiccator.

2.4. Characterization of the 45S5 bioactive glass scaffolds

The microstructure of the scaffolds was characterized using a JSM-6100 JEOL scanning electron microscope (JEOL, Tokyo, Japan) and an X-Tek HMXST 225 X-ray μCT (Nikon Metrology, Tring, UK). Before and after the bioactivity tests, the scaffolds were characterized by X-ray diffraction (Bruker AXS D8 Discover X-Ray Diffractometer) to determine the crystalline phases after sintering and the evolution of the hydroxyapatite layer, respectively. The acquisition data were obtained in the range of 20–90° in 2θ, using CuKα (λ=0.15405 nm) radiation as the source, with a 0.04° step and 2 s/step. Functional groups of bioactive glass and hydroxyapatite phases were determined in the 45S5 bioactive glass scaffolds through infrared spectroscopy, before and after immersion in SBF. Each spectrum was comprised of 32 independent scans in transmittance, measured at a spectral resolution of 1 cm^{-1} within the 4000–400 cm^{-1} range, in a Bruker Tensor 27 FT-IR Spectrometer (Bruker, Germany).

For unconfined compression tests, experiments were conducted using a minimum of 10 randomly selected scaffolds (10 mm in diameter and 5–9 mm in height), which were tested in a universal MTS machine with a cell load of 5 kN. The cross-head loading speed was set at 2.5 mm/min.

The density of the foams was calculated using the mass and dimensions (diameter and height) of the sintered cylinders. The average density was calculated from 40 samples.

3. Results and discussion

The final scaffold had a low-density, open-cell structure. The average density calculated from their dimensions and weight was 1.04 ± 0.08 g/cm^3. The porosity was 61.44 ± 3.13%, and the volume decreased by approximately 25% of the initial volume, after debinding and sintering (Fig. 1). After foaming, the cylindrical foams were

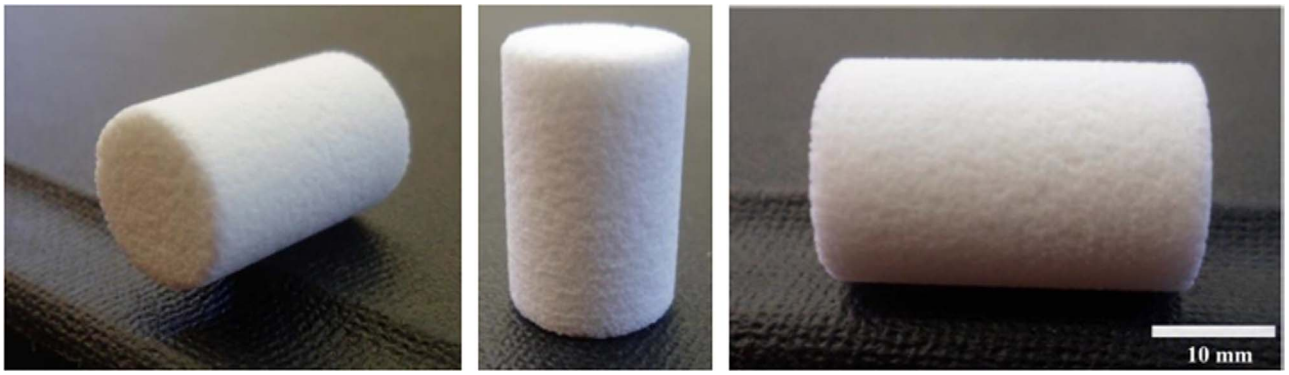

Fig. 1. 45S5 bioactive glass-ceramic scaffolds produced by the powder technology approach.