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Fabrication and characterization of sol–gel derived 45S5 Bioglass®–ceramic scaffolds

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ABSTRACT

Although Bioglass® has existed for nearly half a century its ability to trigger bone formation and tuneable degradability is vastly superior to other bioceramics, such as SiO_2–CaO bioactive glasses. The sol–gel process of producing glass foams is well established for SiO_2–CaO compositions, but not yet established for 45S5 composites containing Na_2O. In this work the sol–gel derived 45S5 Bioglass® has for the first time been foamed into highly porous three-dimensional scaffolds using a surfactant, combined with vigorous mechanical stirring and subsequent sintering at 1000 °C for 2 h. It was found that the mechanical strength of the sintered sol–gel derived Bioglass® scaffolds was significantly improved, attributable to the small fraction of material on the pore walls. More importantly, the compressive strength of the three-dimensional scaffolds produced by this surfactant foaming method could be predicted using Gibson and Ashby's closed cell model of porous networks. A comparative experiment revealed that ion release from the sol–gel derived Bioglass® foams was faster than that of counterparts produced by the replication technique. In vitro evaluation using osteoblast-like cells demonstrated that the sol–gel derived 45S5 Bioglass foams supported the proliferation of viable cell populations on the surface of the scaffolds, although few cells were observed to migrate into the virtually closed pores within the foams. Further work should be focused on modifications of the reaction conditions or alternative foaming techniques to improve pore interconnection.

1. Introduction

Tissue engineering aims at the regeneration of damaged tissue to its natural state, with its primary methodology of using scaffolds populated with signalling molecules and cells [1,2]. In the case of bone tissue engineering two essential features of a scaffold are high porosity (~90%) and appropriate mechanical rigidity [3–5]. Firstly, when cells have attached to the material surfaces there must be enough space and sufficient channels to allow for nutrient delivery and waste removal. Secondly, the scaffold should be able to temporarily replace the mechanical function of damaged bone until sufficient new bone tissue has formed.

Highly porous foams made of ceramics can be fabricated by a variety of processes [6–14]. Among them, foaming sol–gel derived bioactive glasses made by addition of a surfactant combined with mechanical stirring produce scaffolds of high porosities. Currently sol–gel derived bioactive glasses have more simple compositions than 45S5 Bioglass®, and exhibit high bioactivity due to the mesoporous texture inherent in the sol–gel process [15]. In sol–gel foams a hierarchical structure has been achieved with macro-scale pores of diameter >500 μm connected by pore windows [16]. Most work to date has been carried out on the 70S30C composition (70 mol.% SiO_2 and 30 mol.% CaO). The mechanical strength of sol–gel derived 70S30C glass foams has been improved by increasing the sintering temperature (the final stage of foam synthesis) [17]. It has been shown that compressive strength increases from 0.36 to 2.26 MPa when annealing temperatures are increased from 600 to 800 °C, and that increasing the annealing temperature does not have a great impact on the connectivity of the macropore network [17]. The improved mechanical strength of 70S30C foams has been attributed to the formation of a crystalline phase of calcium silicate $CaSiO_3$ [17].

One of the concerns associated with heat treating bioactive glasses at their crystallization temperatures is the possibility of affecting the biodegradability of the scaffold, as a satisfactory degradability is another essential feature of a tissue engineering scaffold. It has been found in previous work [18] that a mechanically competent crystalline phase ($Na_2Ca_2Si_3O_9$) (formed in 45S5 Bioglass® during sintering at 1000 °C) could transform to a degradable amorphous calcium phosphate when the scaffold is incubated in an aqueous solution similar to biological fluids. However, no similar transformation has been reported to take place in

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the crystallized 70S30C composition system [19]. Hence, the primary objective of this study was to produce scaffolds from sol–gel derived 45S5 Bioglass®–ceramics, based on previously reported formulations [16], and carry out a careful investigation of their bioactivity, mechanical properties and cytocompatibility.

A further challenge in the design of bone tissue engineering scaffolds is that a high porosity does not necessarily guarantee restoration of the vasculature of the engineered tissue, which is one of the major obstacles to successfully realizing the clinical use of in vitro engineered tissue and organ substitutes [20]. The ideal scaffold, which will also promote vascularization in vivo, has not yet been determined. Indeed, the major disadvantage of most scaffolds reported to date is that cells tend to adhere only to the outer layer of the scaffolds [21]. This may partially explain why most scaffolds fail to vascularize, independent of their material properties [20]. Therefore, another objective of this work was to carry out an in vitro evaluation of cell infiltration of the sol–gel derived 45S5 Bioglass®–ceramic scaffolds, which will be a useful indication of in vivo tissue penetration into the scaffolds.

2. Experimental procedures

2.1. Foam synthesis

Foams were prepared using a sol–gel derived 45S5 Bioglass® composition (Fig. 1) as described elsewhere [16,22]. Sol preparation involved mixing of the reagents in the following order: deionized water, 2 N nitric acid, tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP), sodium nitrate, and calcium nitrate (all from Sigma–Aldrich). The molar ratio of water to the rest of the chemicals (*R* ratio) was 10:1. A volume of 50 ml of sol was foamed by vigorous agitation with the addition of 0.5 ml of Teepol® (Thames Mead Ltd.) and 1.5 ml of 5 vol.% HF (a gelation catalyst). Teepol® is a detergent containing a low concentration mixture of anionic (15%) and non-ionic surfactants (5%). As the gelling point was approached the foamed solution was cast into cylindrical polymethylpropylene moulds. The samples were aged, dried, thermally stabilized at 600 °C and furnace cooled. The above procedures are summarized in Fig. 1. The above foams were then sintered at 1000 °C for 2 h.

2.2. Characterization

The density ρ_{foam} of the scaffolds was determined from the mass and dimensions of the sintered bodies. The porosity p was then calculated as:

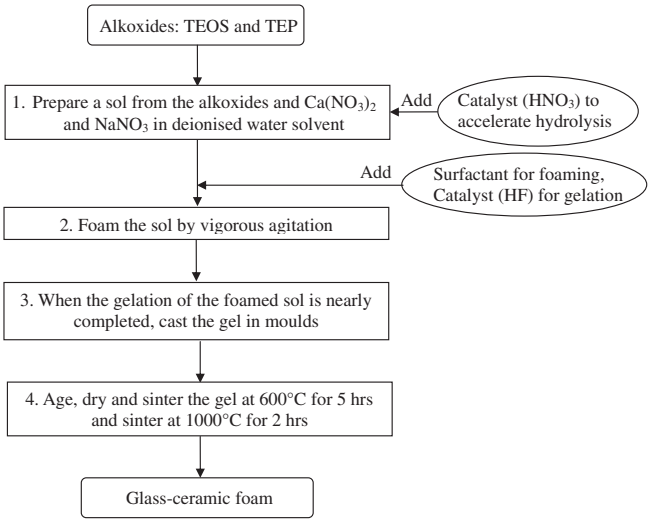

Fig. 1. Flowchart of the production of sol–gel derived Bioglass® foams.

$$p = 1 - (\rho_{foam}/\rho_{solid}) = 1 - \rho_{relative} \quad (1)$$

where $\rho_{solid} = 2.7\ g\ cm^{-3}$ is the theoretical density of sintered 45S5 Bioglass® [23].

The microstructure of the foams before and after immersion in Dulbecco's modified Eagles's medium (DMEM) (tissue culture solution used for biocompatibility studies, outlined below) was characterized by field emission gun (FEG) scanning electron microscopy (SEM) (JEOL 7001). Samples were gold coated and observed at an accelerating voltage of 15–20 keV. Energy dispersive X-ray (EDX) spectra (K_α line) were collected at 20 keV, then processed using an INCA (Oxford Instruments) program, using standard reference spectra.

Selected foams were also characterized using X-ray diffraction (XRD) analysis with the aim of assessing the crystallinity after sintering and the formation of hydroxyapatite (HA) crystals on strut surfaces after different times of immersion in DMEM. The foams were first ground to a powder. Then 0.1 g of the powder was collected for XRD analysis. A Philips PW 1700 Series automated powder diffractometer was used, employing Cu K_α radiation (at 40 kV and 40 mA) with a secondary crystal monochromator. Data were collected over the range $2\theta = 10–60°$ using a step size of 0.04° and a counting time of 25 s per step.

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) analysis was conducted for the Bioglass®–ceramic powders, using a Nicolet 6700 spectrometer and a Smart Orbit single bounce diamond ATR accessory to analyse the chemical bonds in the Bioglass®–ceramics. The data were collected in the range 500–2000 cm^{-1} wave number, at the standard resolution of 0.09 cm^{-1}. The data are presented as is, without "ATR correction", and so no account was made for the wavelength-dependent depth of penetration of the FTIR beam. Due to the intimate contact of the material with the diamond crystal surface the FTIR spectra did not require the use of an internal standard to allow quantitative comparison.

2.3. Mechanical testing

The compressive strength of the foams was measured using an Instron 5848 mechanical tester at a cross-head speed of 0.5 mm min^{-1} and with a 1 kN load cell. The samples were rectangular in shape, with normal dimensions: 20 mm in height and 10 × 10 mm in cross-section. During compression testing the load was applied until densification of the porous samples started to occur.

2.4. Assessment of bioactivity in DMEM

The size of all samples for these tests was 10 × 10 × 10 mm or 10 × 10 × 20 mm (for compression strength testing). Three samples were extracted from the DMEM solution after 7, 14, 21 and 28 days. The DMEM was replaced twice a week because the cation concentration decreased during the course of the experiments, as a result of changes in the chemistry of the samples. Once removed from the incubation fluid the samples were rinsed gently, first in pure ethanol and then using deionized water, and left to dry at ambient temperature in a desiccator.

2.5. Measurement of pH values and ion concentrations

Samples (1 g) of the materials to be tested were soaked in 10 ml of tissue culture medium in 15 ml conical tubes under standard cell culture conditions within a culture incubator (37 °C in humidified air containing 5% CO_2). Acidity was measured at 4, 12, 24, 36 and 48 h. This was followed by replacing the medium with fresh DMEM, which was subsequently incubated for another 48 h.

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