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Development and characterization of lithium-releasing silicate bioactive glasses and their scaffolds for bone repair

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ABSTRACT

Incorporation of therapeutic ions into the structure of bioactive glasses for direct stimulation of cells is a very attractive approach for tissue engineering strategies. Lithium has recently been identified as a biologically active ion which can stimulate osteoblast cell activity. In this study, lithium-containing bioactive glasses (Li-BGs) where Li_2O substitutes Na_2O in different amounts (2.5, 5 and 10 wt.% Li_2O) in 45S5 bioactive glass (BG) were produced and made into 3D scaffolds by the foam replica method. The structural changes that occur after heat treatment, the effect of lithium-content on the bioactivity of the glasses and their lithium ion release profiles were investigated. The results show that the novel Li-BG formulations exhibit the formation of an apatite-like layer on their surface after immersion in SBF for 1 day thus confirming their high surface reactivity, similar to undoped 45S5 BG. XRD results showed that the Li-doped BGs crystallize mainly to combeite ($Na_2Ca_2Si_3O_9$) and silicorhenanite ($SiO_4(PO_4)_2Ca_5$) but also $Li_6P_6O_{18}$ and Li_3PO_4 phases were detected. In terms of lithium ion release, the formulations at 2.5 wt.% and 5 wt.% Li_2O content were the only ones to be within the therapeutic range (<8.3 ppm).

1. Introduction

Bioactive glasses (BGs) are a class of biocompatible and bioactive materials developed to aid bone repair and regeneration, which are based on amorphous silicate compositions. 45S5 Bioglass® (composition in wt.%: 45 SiO_2, 24.5 CaO, 24.5 Na_2O, 6 P_2O_5) is the most investigated BG formulation that was developed by Hench et al. in 1969 [1] and has been demonstrated to be bioactive and osteoconductive [2]. Bioactive glasses have been in clinical use for more than 20 years, mainly in dental applications, as coatings in orthopedic implants or as bone fillers [1–3]. It is a well-known fact that Bioglass® is able to form stable bonds to both soft and hard tissues due to the precipitation of a carbonated hydroxyapatite (HAC) layer on the surface of this material upon contact with relevant body fluids. This phenomenon arises from dissolution and precipitation reactions occurring at the glassy surface resulting in a local increase in the concentration of various key ions that have a marked effect on the metabolism and proliferation rates of osteoblasts [4]. Dissolution products from 45S5 Bioglass® have also been shown to induce osteoblastic differentiation in mesenchymal stem cells (MSCs) by direct stimulation of relevant genes [5]. In the search for improving the biochemical properties of BGs for bone tissue engineering applications, different metallic ions have been incorporated into different glass compositions to stimulate healthy bone formation by triggering the upregulation of desired cellular and tissue processes [6] such as stimulation of angiogenesis by copper [7] or cobalt [8], or suppression of osteoclast activity in the presence of strontium ions [9], while circumventing the use of other delicate and expensive alternatives, such as the incorporation of drugs or growth factors on the BG surface.

Lithium (Li) compounds, such as lithium carbonate, have been in clinical use as mood stabilizing drugs and have been proven to be safe for human use within a maximum recommended therapeutic oral dosage of 1950 mg and 3900 mg of lithium carbonate and citrate respectively per day [10]. It is thought that this effect arises from the enhanced remyelination of peripheral nerves [11] and increased proliferation of neural progenitor cells [12] through the activation of the Wnt/β-catenin signaling pathway following treatments with lithium compounds. Activation of this pathway through cell exposure to Li has been reported to promote osteoblastogenesis and inhibit osteoclastogenesis, leading to an increase in bone formation and volume in rats [13,14]. The effects of lithium on bone density have been investigated, with some authors observing that it might interfere with calcium transport within the body [15], enhance the proliferation, differentiation and

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cementogenic gene expression in human periodontal ligament-derived cells [16] and osteogenic differentiation in mesenchymal stem cells (MSCs) [17]. A study investigating the effects of lithium in patients treated with lithium carbonate found that such treatment may enhance or preserve bone mass [18]. Recently, lithium substituted 45S5 Bioglass® with different concentrations of Li_2O were investigated and increased proliferation rates and alkaline phosphatase activity of osteoblastic cells were reported [19]. Such preliminary results on melt-derived (glassy, amorphous) 45S5 BG samples motivated the present research to further investigate Li-doped 45S5 bioactive glass (Li-BG), focusing however, on sintered and crystallized structures relevant for scaffold fabrication by the foam replica method [20].

Therefore in this study, different amounts of Li_2O substitute Na_2O in the original Bioglass® formulation to obtain Li-BGs at 2.5, 5 and 10 wt.% Li_2O. The goal was thus to produce and characterize novel compositions of lithium containing bioactive glass powders and to investigate the fabrication of 3D scaffolds with Li-releasing ability which should exhibit properties suitable for bone tissue engineering. The effect of lithium on the reactivity of the glass was investigated by immersing samples in SBF and observing the morphological and structural changes related to hydroxyapatite (HA) deposition on the scaffold surface and investigating lithium ion release.

2. Experimental methods

2.1. Materials

Sodium carbonate (Na_2CO_3 Merck, Germany), ≥99% purity, calcium phosphate ($Ca_3(PO_4)2$, Sigma-Aldrich, Germany), ≥96% purity, lithium carbonate (Li_2CO_3, Merck, Germany), ≥99% purity, silicon oxide (SiO_2, Merck, Darmstadt, Germany), and calcium carbonate ($CaCO_3$, Merck, Germany) were used. The as-received powders were weighed and mixed to obtain three different formulations of lithium-containing bioactive glasses (Li-BGs) in 300 g batches of compositions shown in Table 1. The substitution of Na_2O by Li_2O was done on a wt.% basis rather than the more appropriate mol.% basis, to be able to compare results with those of a previous study [19]. The homogenized powders were melted in Pt crucibles at 1400 °C for 1 h. These processing parameters were chosen following the mentioned study of Khorami et al. [19]. The melt was then quenched rapidly in water and then the frit was crushed into a rough powder using a Jaw Crusher (Retsch, Germany) and subsequently ground into a fine powder using a zirconia planetary ball mill (Retsch, Germany) to obtain a fine powder with a mean particle size of 2–5 μm. Commercially available bioactive glass of 45S5 composition with a mean particle size of <5 μm (Schott, Germany) was used as a base material to compare the properties of the Li-BGs.

2.2. Sintering monitoring

The densification of the new Li-BGs was characterized using a range of techniques.

Differential scanning calorimetry (DSC) was carried out to detect phase transitions as a function of temperature. Thermograms were recorded from room temperature to 1300 °C using a DSC404 F1 Netzsch high temperature differential scanning calorimeter (Netzsch, Germany). A sample of at least 22 mg was heated up in air at a rate of 10 °C/min. The samples were placed on a Pt/Rh crucible to ensure an efficient heat transfer. Thermograms were corrected to compensate for the normal variation of the equipment setup by baseline correction. The glass transition temperature was determined at the onset of the transformation.

Cylindrical BG pellets with a diameter of 1.3 cm were prepared by pressing powders without adding any binder at a pressure of 50 MPa using a press die. The pellets were placed in the heating chamber of an optical dilatometer (Fraunhofer, Germany). This technique was used to evaluate possible anisotropy during shrinkage of the Li-BGs samples, one sample per composition was tested. The samples were sintered in an inert atmosphere (Argon) at approximately 1 °C/min up to 1050 °C following the same sintering program normally used to obtain 45S5 BG scaffolds [20]. To record the sintering process, two images of the sample were taken every minute. Measurements of height and width of the samples were recorded and the volume of the samples was calculated and normalized and changes to these dimensions were recorded in every image by the equipment software.

The sintering behavior of the Li-BGs was also investigated by heating glass powder samples at a constant rate in an environmental scanning electron microscope (E-SEM) to observe the *in-situ* morphological changes of the glass particles as a function of temperature. Very small amounts (a few particles) of the powdered bioactive glasses were placed in a platinum-coated ceramic crucible of 7mm in diameter. All measurements were carried out in a FEI/Philips Field Emission Gun Environmental Scanning Electron Microscope (FEG-ESEM) XL-30. The samples were heated at 5 °C/min up to 1100 °C at 1.9 Torr, stopping to acquire images of the samples every few degrees.

2.3. Scaffold fabrication

Cubic scaffolds with approximate dimensions of 7 mm × 7 mm × 7 mm mm were prepared by the foam replica technique as described by Chen et al. [20]. Cubic polyurethane (PU) foams (45 ppi, Eurofoam) were immersed in an aqueous slurry containing 1 g/ml BG powder and 0.01 mol/ml polyvynil alcohol (PVA) under vigorous stirring for 1 min. The sponges were then removed from the slurry and any excess was squeezed out. This process was repeated once more and the resulting green bodies were dried overnight at 60 °C and then sintered according to the characteristic temperatures of each glass (see Table 2), namely glass transition (T_g), onset crystallization ($T_{o,c}$), crystallization (T_c) and melting peak (T_m) temperatures.

2.4. Scaffold bioactivity and degradation

The bioactivity of the Li-BGs was investigated by immersing sintered scaffolds (7 mm × 7 mm × 7 mm) in simulated body fluid (SBF) for up to 14 days and examining the deposition of hydroxyapatite (HA) on their surface. Samples were immersed in 50 ml of SBF, prepared according to Kokubo et al. [21], and placed in an orbital shaker (KS 4000i control, IKA®) at 37 °C and 90 rpm. The volume of solution used was in agreement with existing literature [20] and it was not exchanged throughout the duration of the study to examine the ionic release in solution.

Table 1
Composition of batches for Li-BG preparation.

	SiO_2/wt.%	CaCO /wt.%	$CaPO_4$/wt.%	Na_2CO /wt.%	Li_2CO /wt.%
2.5% Li-BG	45	31.03	13.11	37.62	2.5
5% Li-BG	45	31.03	13.11	33.34	5
10% Li-BG	45	31.03	13.11	24.79	10

Table 2
Characteristic temperatures of Li-BGs determined by DSC.

wt.% Li	T_g/°C	$T_{o,c}$/°C	T_c/°C	T_m/°C	$T_{o,c} - T_g$/°C
0	530	605	680	1217	75
2.5	507	590	650	1180	83
5	490	587	630	1154	97
10	480	604	624	1100	124

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