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Abstract

Biocompatible implantable glasses doped with rare earth elements (e.g. holmium) are regarded as promising biomaterials for advanced medical therapies. In this work, Ho-containing scaffolds based on the 58S sol-gel glass composition were fabricated for the first time through sponge replication followed by sinter-crystallization, which was associated to the formation of $CaSiO_3$. The obtained materials exhibited high porosity (86 vol.%), trabecular pore-strut 3D architecture and compressive strength (around 1 MPa) comparable to that of osseous tissue, making them promising candidates for use in bone applications.

Keywords: Biomaterials; Bioactive glass; Porous materials; Holmium

1. Introduction

Bioactive glasses are clinically used for a wide range of medical applications especially in contact with bone tissue, mainly in orthopaedics and dentistry [1]. Doping bioactive glasses with metallic elements is a valuable approach to impart special extra-functionalities that are commonly activated upon the release of ionic species in the body. Some examples are silver for inducing antibacterial effects [2] and copper for promoting angiogenesis [3]; less-common ions incorporated in bioactive glasses were recently reviewed in [4].

Bioactive glasses have also been proposed as radioisotope vectors to be applied in the treatment of some types of cancer; as sol-gel glasses are typically soluble, special care should be addressed to tailor the release kinetics of radionuclides in order to be within the safety limits recommended for brachytherapy treatment, as discussed in [5]. Such glasses are typically doped with rare earth elements that, after neutron activation, become short-range and short-half-life β-ray emitters to kill cancer cells locally. In this regard, ^{166}Ho emits high-energy β-rays that penetrate about 8.4 mm into the body tissue, reducing the side effects to healthy cells in the treated area [6]. Delpino et al. [7] recently synthesized a set of 58S-based sol-gel glasses containing 1.25 to 5 wt.% Ho and reported that all compositions exhibited apatite-forming ability upon immersion in SBF, thus being suitable for bone regeneration; furthermore, the glass doped with 5 wt.% Ho even stimulated the viability of pre-osteoblastic cells. The same Ho-doped glass powders were also incorporated into poloxamer-407 hydrogel to produce anticancer bioactive composites able to selectively kill osteosarcoma cells [8]. In light of these promising findings, the present study aims to explore, for the first time, the feasibility of Ho-containing glass-derived 3D scaffolds.

2. Materials and methods

2.1 Glass synthesis and characterization

Ho-containing 58S-based glass (5Ho58S; composition: 95($58SiO_2$-$33CaO$-$9P_2O_5$)-$5Ho_2O_3$ wt.%) was synthesized by a quick-alkali sol-gel route, as described in a previous work [7]. Shortly, tetraethyl orthosilicate (TEOS, 99.99 %, Sigma Aldrich) and triethyl phosphate (TEP, >99.8 %, Sigma Aldrich) were added to a solution containing deionized water, ethanol and 2 M HNO_3 (Sigma-Aldrich) in a 13.9:50:2 ratio, and mixed under magnetic stirring for 20 min. Then, $Ca(NO_3)_3{\cdot}4H_2O$ (>99.0 %, Sigma Aldrich), and $Ho(NO_3)_3{\cdot}5H_2O$ (99.9 %, Sigma Aldrich) were added to the previous acidic solution and stirred for further 20 min. Afterwards, 10 mL of 2 M ammonia solution (20-30%, Sigma Aldrich) were quickly dropped (by <2 s) into the batch to induce gelation. The gel was freeze-dried (Operon, South Korea) for 24 h and calcined at 550 °C for 1 h in air (Furnace EGD 300, Brazil). The so-obtained 5Ho58S powder was ball milled (Pulverisette 0, Frtisch, Germany) and sieved to a final particle size <32 μm. The distribution of 5Ho58S powder size was assessed by a particle size analyser (LS230, Beckam Coulter Corporation, Indianapolis, IN, USA).

5Ho58S powders were characterized by hot-stage microscopy (HSM; Expert System Solution, Modena, Italy) and differential thermal analysis (DTA; DTA404PC, Netzsch, Germany) in air to analyse thermal behaviour. For HSM, small disks of compacted powder (diameter 1 mm, height 3 mm) was placed onto an alumina tile; then, black silhouettes of the sample were taken from 20 °C to the melting point (heating rate 10 °C/min). The variation of the sample dimensions upon the heating cycle was measured in terms of height of the silhouettes. DTA was performed from 20 to 1400 °C (heating rate 10 °C/min) using alumina powder as a reference; characteristic temperatures were obtained directly from the thermogram.

5Ho58S powder also underwent X-ray diffraction (XRD; X'Pert diffractometer, source voltage 40 kV and current 30 mA, Bragg-Brentano camera geometry, Cu Kα radiation with $\lambda = 15.405$ nm, step size 0.02°, counting time per step 1 s) in the

2θ-range from 10 to 70°; crystalline phases were identified through X'Pert HighScore software equipped with PCPDFWIN database.

2.2 Scaffold fabrication and characterization

Scaffolds were produced by foam replication using a 45-ppi polyurethane sponge as a sacrificial template; the method was described in detail elsewhere [9]. Shortly, porous polyurethane cuboids (side 10 mm) were dipped into a water-based 5Ho58S-containing suspension (solid load : distilled water : poly(vinyl alcohol) (binder) = 15:79:6 (wt.%)). After being withdrawn from the slurry, the polymeric sponge was compressed for 6 mm along three orthogonal directions to homogeneously squeeze the suspension out of the pores. This dipping-squeezing cycle was repeated three times for each sample, which was then dried overnight in ambient conditions and thermally treated at 1050 °C for 3 h (heating rate 10 °C/min) in order to burn off the organic template and sinter the 5Ho58S particles, thereby obtaining a positive replica of the sacrificial polymer. It is worth mentioning that 58S glass scaffolds have been typically produced by sol-gel foaming in the past [10]; in this work, sponge replication was adopted to shorten fabrication time and avoid the use of HF as a catalyst.

5Ho58S-derived scaffolds were sputter-coated with chromium and their morphology and 3D porous architecture were investigated by field-emission scanning electron microscopy (FESEM, SupraTM 40 Zeiss, Germany; accelerating voltage 3 kV).

Powdered scaffold underwent XRD under the same experimental conditions used for analysing as-such 5Ho58S particles.

Total porosity of scaffolds was estimated as $(1 - \rho_s/\rho_0)\times100$, where ρ_s is the scaffold density (mass-to-volume ratio) and ρ_0 is the density of non-porous glass.

Scaffolds (8 mm × 8 mm × 8 mm cuboids) were mechanically tested by crushing test (MTS Model 43, MTS, Eden Prairie, MN, USA; cell load 44 kN; cross-head speed 1 mm/min). The compressive strength was calculated as the ratio between the peak load registered during the test and the resistant cross-sectional area of each sample. Results of porosity and compressive strength were expressed as mean ± standard deviation after quintuplicate measurements.

3. Results and discussion

The particle size distribution of 5Ho58S particles is shown in Figure 1a; the average particle size was 22 μm. Glass transition temperature (T_g = 625 °C), crystallization onset (T_x = 850 °C) and maximum rate of crystallization (T_c = 890 °C) of 5Ho58S were determined from DTA plot (Figure 1b). The exothermic peak centred at T_c can be associated to the formation of wollastonite, as suggested by both the 5Ho58S composition and the thermal range for the development this crystalline phase (850-950 °C); this was also confirmed by XRD analysis (Figure 2).

HSM curve (Figure 1c) revealed a double-stage shrinking behaviour described by the temperatures related to first shrinkage (T_1 = 700 °C), end of first shrinkage (T_2 = 890 °C), second shrinkage (T_3 = 1130 °C), end of second shrinkage (T_4 = 1280 °C). Softening (T*) just starts at the end of the thermal cycle. First and second shrinkages were quantified to be around 20 and 15%, respectively. The sintering temperature for scaffolds (1050 °C) was selected around the mid of the first-densification plateau. The competition between sintering and crystallization can be described by the parameter S_c (sinterability), first introduced by Lara et al. [11] and defined as the difference between crystallization onset (T_x) and temperature of maximum shrinkage (in this case T_4). Hence, we have $S_c = T_x - T_4 < 0$, meaning that only partial densification is achieved before crystallization begins, i.e. there is no sintering temperature that allows complete densification of 5Ho58S powders avoiding concurrent crystallization phenomena.

5Ho58S is an amorphous material, as revealed by the broad halo in the 25-35° 2θ-range which is typical of silicate glasses (Figure 2a), while pseudowollastonite ($CaSiO_3$, code 00-089-6485), was detected as crystalline phase in the scaffold (Figure 2b), which is consistent with DTA results. $CaSiO_3$ is a highly biocompatible phase as revealed by in vivo studies [12].

SEM images (Figure 3a,b) show that the sintered scaffold successfully replicated the structure of the polyurethane template, which is similar to cancellous bone [9]; the macropores are well interconnected and their size is well above 100 μm, as recommended for bone applications to allow biofluid perfusion and cell colonization [13]. SEM images at higher magnification (Figure 3c,d) reveal the presence of a micrometric or even submicronic roughness on the surface of scaffold struts, which can be beneficial to promote cell attachment [14].

The total porosity (86.1 ± 3.5 vol.%) of scaffolds is potentially suitable for bone applications (recommended value: >50 vol.% [13]) and comparable to that of other biomedical foams based on sol-gel 58S glass [15] or melt-derived silicate compositions (e.g. 45S5 [16] or 13-93 glass [17]).

The compressive strength of 5Ho58S-derived scaffolds was 1.1 ± 0.2 MPa, which is within the typical range of human cancellous bone (0.1-16 MPa [18]) and higher than that exhibited by other foams reported in the literature, like 45S5 Bioglass® (0.27-0.42 MPa, porosity 89 vol.%) [19] and dopant-free 58S scaffolds (0.16 MPa, porosity 89 vol.%) [15]. The significantly lower strength of 58S scaffolds reported in [15] as compared to the 5Ho58S ones produced in the present work can be attributed to the lower sintering temperature (700 °C), yielding to less-densified struts.

4. Conclusions

This work demonstrates the feasibility of using foam replication to make Ho-containing 58S glass-derived scaffolds that exhibited a 3D network of open macropores (total porosity 86 vol.%, pore size >100 μm) mimicking the trabecular

architecture of cancellous bone. Furthermore, the scaffolds had a compressive strength of 1.1 ± 0.2 MPa. These results suggest the potential suitability of the scaffolds for advanced bone applications, like bone cancer brachytherapy combined with bone regeneration, and motivate further investigation on this topic.

CRediT authorship contribution statement

All authors contributed to conceptualization, methodology, investigation and writing of the paper.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure legends

Figure 1. Analysis of 5Ho58S powders: (a) particle size distribution and thermal analyses – (b) DTA and (c) HSM plots.

Figure 2. XRD patterns of (a) 5Ho58S powder and (b) scaffold (sintered at 1050 °C/3 h and subsequently ground in powder).

Figure 3. SEM micrographs of 5Ho58S-derived scaffold: (a) pore-strut architecture (magnification 150×), (b) detail of a macropore (500×), (c) and (d) surface at different magnifications (2,000× and 20,000×).