Materials Letters 130 (2014) 281–284

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

Zirconia-containing radiopaque mesoporous bioactive glasses

Francesca Tallia [a,1], Marta Gallo [b,2], Lucia Pontiroli [a], Francesco Baino [b], Sonia Fiorilli [b], Barbara Onida [b], Giovanni C. Anselmetti [c], Antonio Manca [d], Chiara Vitale-Brovarone [a,b,*]

[a] Bionica Tech S.r.l., Corso G. Sommeiller 32, 10128 Torino, Italy
[b] Dipartimento di Scienza Applicata e Tecnologia, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy
[c] GVM-Care & Research—Villapia Hospital, Strada Comunale di Mongreno 180, 10132 Torino, Italy
[d] Radiology Unit, Istituto di Candiolo—IRCCS, Fondazione del Piemonte per l'Oncologia, Strada Provinciale 142 km 3.95, Candiolo, 10060 Torino, Italy

ARTICLE INFO

Article history:
Received 11 February 2014
Accepted 10 May 2014
Available online 17 May 2014

Keywords:
Radiopacity
Mesoporous bioactive glass
Zirconia
Sol–gel preparation
Bone tissue engineering
Injectable cements

ABSTRACT

A radiopaque mesoporous bioactive glass (named MBGZ-7) was obtained through a combined sol–gel and evaporation induced self-assembling (EISA) route, adding zirconium propoxide to the synthesis batch as the zirconia precursor. The nitrogen sorption analysis confirmed the mesoporous nature of the glass. The assessment of in vitro bioactivity by soaking in acellular simulated body fluid (SBF) and SEM observation showed the deposition of hydroxyapatite crystals on its surface after one week. The good radiopacity level was demonstrated by comparing X-ray images of MBGZ-7 and a blank sample that did not contain radiopaque additives. It is envisaged the use of MBGZ-7 as a promising dispersed phase in composite materials for minimally invasive surgery procedures, such as injectable bone cements, in order to allow the visualization of the implant under fluoroscopic control, during both injection and follow-up.

1. Introduction

Radiopacity is an essential feature of bone cements, in order to allow an easy follow-up of the treated patient and, in case of mini-invasive procedures such as vertebroplasty, to allow the injection of the material under fluoroscopic control [1]. Traditionally, the radiopacity of cements is gained through the addition of a radiopaque agent, such as particles of barium sulfate ($BaSO_4$), zirconia (ZrO_2) or iodine-based organic molecules. This particulate dispersed phase has been proven to cause a worsening of the mechanical properties of the cement and to activate macrophages, thereby contributing to bone resorption [2,3]; in the case of release of the iodine-based monomer, a toxic effect on living organisms has also been observed [4,5].

In order to overcome all these drawbacks, we developed an inherently radiopaque mesoporous bioactive glass meant to be used as a dispersed phase in injectable bone cements, avoiding the use of additional radiopaque particles. The addition of bioactive glass or glass–ceramic particles in bone cements is well known in literature for their ability to bond to the bone tissue [6,7], as discovered by Hench [8,9]; moreover, the interest on mesoporous bioactive glasses (MBGs) is growing fast because of their reported osteoinductive ability and their drug loading potential [10–13].

Herein, for the first time to the best of the Authors' knowledge, radiopacity is gained through the addition of zirconia (ZrO_2) into a mesoporous glass network, maintaining satisfactory bioactive properties. The production of mesoporous zirconia is widely described in literature [14–16] and, recently, Zhu et al. [17] produced Zr-incorporated MBGs scaffolds using zirconium tetrachloride as the zirconium precursor. We combined the work of Yan et al. [10] on the sol–gel synthesis of MBGs with the work of Liu et al. [15] on mesoporous zirconia in order to produce Zr-containing mesoporous bioactive glasses (named MBGZ-7 and MBGZ-15) using zirconium propoxide as the zirconia precursor. The invention of this novel class of radiopaque mesoporous glasses and their future applications are disclosed in a patent application recently deposited by Vitale-Brovarone et al. [18].

2. Materials and methods

Basing on the sol–gel synthesis described by Yan et al. [10], MBGZ-7 was synthesized by using the commercial non-ionic block copolymer Pluronic P123 ($EO_{20}PO_{70}EO_{20}$, where "EO" is poly

* Corresponding author at: Dipartimento di Scienza Applicata e Tecnologia, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy. Tel.: +39 011 0904716.
E-mail address: chiara.vitale@polito.it (C. Vitale-Brovarone).
[1] Department of Materials, Imperial College London, South Kensington Campus, Prince Consort Road, SW7 2BP London, United Kingdom.
[2] Université de Lyon, INSA-Lyon, Université Lyon 1, MATEIS CNRS UMR 5510, 7 Avenue Jean Capelle, 69621 Villeurbanne Cedex, France.

http://dx.doi.org/10.1016/j.matlet.2014.05.062
0167-577X/

(ethylene glycol) and "PO" is poly(propylene glycol)) as an organic surfactant, which acts as structure-directing agent for pores formation. In a typical synthesis of MBGZ-7 ($SiO_2/CaO/P_2O_5/ZrO_2$=73:15:5:7 molar ratio), a synthesis batch was prepared by dissolving P123 (4.0 g), tetraethyl orthosilicate (TEOS, 6.10 g), calcium nitrate tetrahydrate ($Ca(NO_3)_2\cdot 4H_2O$, 1.42 g), triethyl phosphate (TEP, 0.73 g), zirconium propoxide (0.92 g), acetylacetone (Acac, which acts as a stabilizer to prevent the zirconium propoxide from uncontrollable hydrolysis and consequent precipitation, 0.10 g [15]) and HCl 0.5 M (1.0 g) in ethanol (60.0 g). All chemicals were purchased from Sigma-Aldrich, Italy, and used as received. This synthesis batch, continuously stirred at 35 °C for 24 h, resulted in a sol that, once casted into Petri dishes, underwent an ageing step (24 h at room temperature followed by 24 h at 120 °C), during which the evaporation-induced self-assembly (EISA) process occurred. The dried gel was calcined at 750 °C for 5 h in air to obtain the final MBGZ-7 product in form of thin membranes, which were then ground and sieved when needed. For comparison, a traditional mesoporous bioactive glass without zirconia (MBG, $SiO_2/CaO/P_2O_5$=80:15:5 molar ratio) was synthesized by an identical process, avoiding the addition of zirconium propoxide and Acac. Another glass composition (referred to as MBGZ-15), in which the zirconia molar percentage was increased up to 15% to the detriment of the silica content, was also prepared maintaining unchanged the other synthesis and process parameters for purpose of comparison.

The obtained powders underwent wide-angle (2θ within 10–70°) X-ray diffraction (XRD) through a Philips X'Pert diffractometer (Bragg-Brentano camera geometry with Cu $K\alpha$ incident radiation; working conditions: 40 kV, 30 mA). Long-range order was assessed through low-angle XRD (2θ within 0.6–5°), whereas specific surface area (SSA) and porosity were characterized by N_2 adsorption/desorption measurements at −196 °C performed using a Quantachrome Autosorb1. BET SSA was calculated in the relative pressure range 0.04–0.1 and the pore size was evaluated through the BJH method on the isotherm desorption branch.

In vitro bioactivity tests were carried out by soaking MBGZ-7 in simulated body fluid (SBF) [19] at 37 °C for 1, 3 and 7 days with refresh of the solution every 48 h to simulate fluid circulation in the human body. After soaking, the samples were dried at room temperature and then investigated through scanning electron microscopy (SEM, FEI Quanta Inspect 200LV) equipped with electron dispersive spectrometer (EDS, EDAX Genesis) for compositional analysis, to monitor the formation of hydroxyapatite (HA) on their surface over time.

X-ray images of MBG, MBGZ-7 and MBGZ-15 powders were taken with Digital Radiography equipment (Philips PCR Eleva, Philips Medical System DMC GmbH, Hamburg—Germany) with exposure parameters of 45 kV and 100 mA and exposure time of 0.4 s. Plain X-rays were post-processed with Osirix software for Mac (Pixmeo SARL, Switzerland). A semi-quantitative analysis of radiopacity was performed drawing a round region of interest (ROI) on the center of each sample with a fixed surface area. The program analyzed the radiopacity of the samples obtaining a whole number that was not influenced by the grayscale visualization window. A single plain X-ray could contain all samples, thus any possible bias due to exposure parameters could not influence the ratio of radiopacity between the different samples.

3. Results

A type IV N_2 sorption isotherm is observed for MBGZ-7 and MBGZ-15 samples, with a hysteresis loop representing the filling of mesopores (Fig. 1). This finding is further supported by low-angle XRD patterns (Supplementary information (SI)—Fig. A),

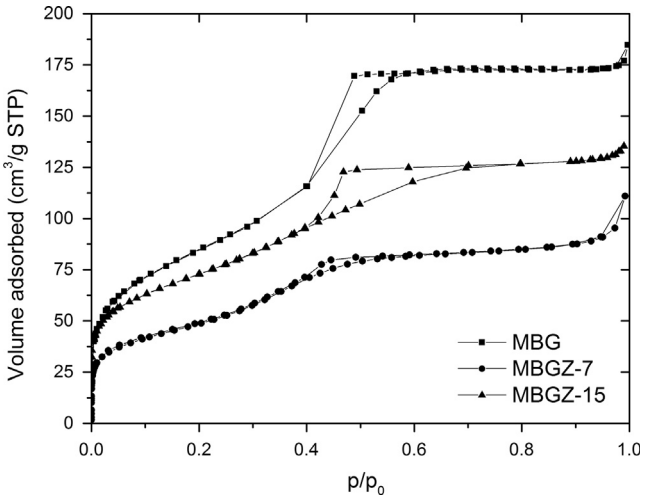

Fig. 1. Nitrogen sorption isotherms of MBG, MBGZ-7 and MBGZ-15.

Table 1
Textural parameters of the synthesized glasses obtained from N_2 sorption isotherms.

Glass	SSA ($m^2\ g^{-1}$)	Vol ($cm^3\ g^{-1}$)	Average d_{BJH} (nm)	d_{XRD} (nm)
MBG	307	185	3.5	6.1
MBGZ-7	174	111	3.3	5.8
MBGZ-15	262	135	3.6	6.5

which show a single low-angle XRD peak (2θ=1.45°, 1.53° and 1.35° for MBG, MBGZ-7 and MBGZ-15, respectively), characteristic of a wormhole structure. A comparison among MBG, MBGZ-7 and MBGZ-15 in terms of specific surface area (SSA), specific volume (Vol), average pore size (d_{BJH}) and XRD basal d-spacing (d_{XRD}), calculated through the Bragg law, is reported in Table 1. A broad halo within the range 2θ=20–30°, typical of amorphous silica, is visible in wide-angle XRD pattern of MBGZ-7 and MBGZ-15 (SI—Fig. B); no signals due to crystalline ZrO_2 phases are observed in any case.

SEM images of MBGZ-7 surface before and after 1 and 7 days of soaking in SBF (Fig. 2a–c, respectively) show the progressive nucleation of HA particles, whose composition is confirmed by the EDS spectrum (Fig. 2d). A quantification of Ca/P ratio is not possible because zirconium and phosphorus peaks are overlapped; however, it is clear that, by incrementing the soaking time, the amount of precipitates increases and the structure of HA changes, determining the formation of HA microcrystals after 1 week (Fig. 2c).

From the X-ray image reported in Fig. 3 it is evident that an increase in zirconia percentage within glass composition determines an enhancement in radiopacity: considering an analogous ROI for all samples (equal to 0.223 cm^2), MBGZ-7 and MBGZ-15 show an increase of 11% and 17% in radiopacity intensity, respectively, if compared to MBG, whose radiopacity is given only by Ca and P and which therefore cannot be distinguished from bone.

4. Discussion

N_2 sorption isotherms (Fig. 1) and low-angle XRD analyses confirmed that mesoporous bioactive glasses containing zirconia were successfully synthesized. The addition of ZrO_2 (up to 15 mol% with respect to oxides ratio) does not affect the peculiar features of the starting glass structure: both MBGZ-7 and MBGZ-15 show an