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Mesoporous bioactive scaffolds prepared with cerium-, gallium- and zinc-containing glasses

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ABSTRACT

Mesoporous bioactive glass scaffolds (MBG_Scs), based on 80% SiO_2–15% CaO–5% P_2O_5 (in mol.%) mesoporous sol–gel glasses substituted with Ce_2O_3, Ga_2O_3 (both 0.2% or 1.0%) and ZnO (0.4% or 2.0%), were synthesized by combination of evaporation-induced self-assembly and rapid prototyping techniques. Cerium, gallium and zinc trace elements were selected because of their inherent beneficial biological properties. Fabricated scaffolds were characterized and compared with unsubstituted scaffold (B_Sc). All of them contained well interconnected ultralarge pores (pores >400 μm) ideal for vascular ingrowth and proliferation of cells. Macropores of size 100–400 μm were present inside the scaffolds. In addition, low-angle X-ray diffraction showed that B_Sc and scaffolds with substituent contents up to 0.4% exhibited ordered mesoporosity useful for hosting molecules with biological activity. The textural properties of B_Sc were a surface area of 398 $m^2\ g^{-1}$, a pore diameter of 4.3 nm and a pore volume of 0.43 $cm^3\ g^{-1}$. A slight decrease in surface area and pore volume was observed upon substitution with no distinct effect on pore diameter. In addition, all the MBG_Scs except 2.0% ZnO_Sc showed quite quick in vitro bioactive response. Hence, the present study is a positive addition to ongoing research into preparing bone tissue engineering scaffolds from bioceramics containing elements of therapeutic significance.

1. Introduction

Bone regeneration is a natural phenomenon by which new bone is formed during the normal remodeling process, as well as after injury. However, there are certain clinical situations where bone cannot heal itself because the defect is too large or the bone has lost its regenerative capabilities. Bone tissue engineering has emerged as a promising technique in such situations, stimulating regeneration of host bone without posing constraints found in gold standard bone grafting methods [1,2]. It utilizes three-dimensional porous biomaterial scaffolds which act as a temporary framework providing a suitable environment for normal cell growth, and hence helps in tissue regeneration [3].

The success of a synthetic scaffold depends on whether it satisfies requirements similar to those found in nature for normal bone development. Natural bones possess hierarchical porosity in the range of 1–3500 μm, which is necessary for several physiological functions [4]. Ideally the template must consist of an interconnected porous structure with ~90% porosity. Pore sizes greater than 100 μm enable cell seeding, tissue ingrowths and vascularisation. Pores in the microporous (<2 nm) or mesoporous (2–50 nm) range promote cell adhesion, adsorption of biological metabolites and resorbability at rates controlled to match that of tissue repair [5–8].

Mesoporous bioactive glass scaffolds have set a mark in the field of bone tissue engineering for exhibiting a well-interconnected macroporous network along with mesopores, enabling them to carry therapeutic drugs [9–12]. Recently, efforts have being made to incorporate elements that have a relevant function and biological significance in the glass matrix [13–16]. This approach is considered to be economical and stable, as such elements do not pose a risk of decomposition during scaffold manufacture [17].

Recently, the beneficial biological features of cerium, gallium and zinc have prompted scientists to study them in different glass systems [18–22]. Studies have shown that cerium has a positive effect on primary mouse osteoblasts in vitro and cerium oxide nanoparticles act as neuroprotective agents [23,24]. In addition, gallium increases bone calcium content, inhibits osteoclast activity and

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shows antimicrobial activity [25–27]. Zinc has a stimulatory effect on bone formation, and also shows antimicrobial activity [28,29].

These studies motivated us to incorporate $x$$Ce_2O_3$, $x$$Ga_2O_3$ or xZnO into sol–gel (BGs) and mesoporous bioactive glasses (MBGs) with the composition $(80 - x)$% SiO_2–15% CaO–5% P_2O_5 (in mol.%) [30,31]. Structural characterization of Ce_2O_3-, Ga_2O_3- or ZnO-substituted BGs by ^{29}Si magic angle spinning nuclear magnetic resonance showed that the glass network connectivity of unsubstituted BGs (75% of Q^4 content) reduced upon the addition of Ce ions, which thus acts mainly as a network modifier, while gallium and zinc ions behave as intermediate ions. On the other hand, experimental results confirmed that MBGs substituted with a low concentration of Ce_2O_3, Ga_2O_3 or ZnO maintained their mesoporous order, high textural properties such as surface area of 462 $m^2\ g^{-1}$, pore size of 4.4 nm, pore volume of 0.49 $cm^3\ g^{-1}$ and the ability to form apatite rapidly in vitro (except xZnO over 2.0%), and that the cerium, gallium and zinc were distributed homogeneously in the glass network. On the other hand, $x$$Ce_2O_3$, $x$$Ga_2O_3$ and xZnO-BGs took ~7–15 days for hydroxycarbonate apatite (HCA) formation, except xZnO over 4.0%.

Preliminary experimental results indicate that Ce-, Ga- and Zn-MBGs possess the optimum properties required for a material to be used for fabrication of scaffolds. Thus, in the present study we took the further step of preparing three-dimensional (3-D) scaffolds from quaternary MBGs substituted with cerium, gallium or zinc elements, which possessed valuable features (see Fig. 1). The focus of the study was to determine if the fabricated scaffolds exhibit hierarchical porosity and maintain the textural properties and in vitro response of MBG powder with the view to recommending them for bone tissue engineering.

2. Materials and methods

2.1. Synthesis of MBG powders

Cerium, gallium and zinc-containing $(80 - x)$% SiO_2–15% CaO–5% P_2O_5 (in mol.%) mesoporous sol–gel glasses, the compositions of which are given in Table 1, were synthesized according to a method described in a previous publication [32] . In the present study, glasses were prepared by replacing a small part of SiO_2 with Ce_2O_3, Ga_2O_3 or ZnO, whereas the amounts of CaO and P_2O_5 were kept constant in order to maintain the optimal Ca/P ratio required for fast bioactive response, as reported in another study on SiO_2–CaO–P_2O_5 sol–gel glasses [33]. During synthesis, Pluronic P123, tetraethyl orthosilicate, triethyl phosphate, calcium nitrate ($Ca(NO_3)_2.4H_2O$) and cerium nitrate, gallium nitrate or zinc nitrate were dissolved in ethanol containing 0.5 N HNO_3. The sol obtained was added to a Petri dish to undergo evaporation-induced self-assembly (EISA) [34] for 7 days. The dried gels were then calcined at 700 °C for 3 h in order to remove surfactant. The glasses thus obtained were subjected to milling to obtain grains of size below 32 μm for mesoporous bioactive glass scaffold (MBG_Sc) preparation.

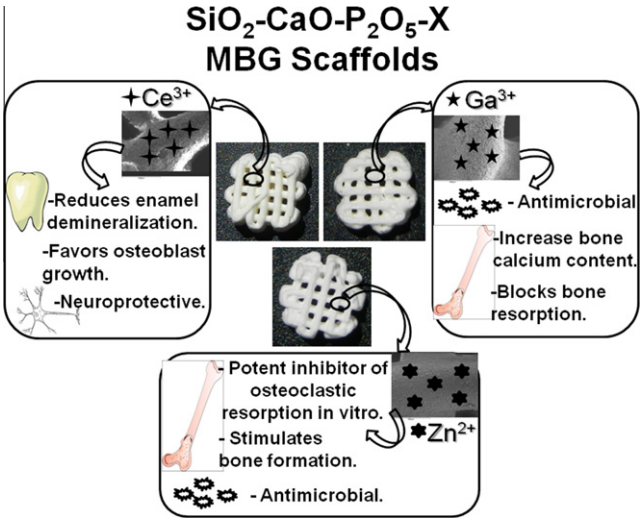

Fig. 1. Schematic representation of possible biological properties possessed by Ce^{3+}-, Ga^{3+}- and Zn^{2+}-substituted MBG_Scs prepared by rapid prototyping: 3-D printing.

2.2. Preparation of MBG scaffolds by rapid prototyping: 3-D printing

The rapid prototyping technique [35] consists of reproducing a previous computer-aided design format by injecting a paste using a robot injector.

2.2.1. Preparation of paste for robot injection

MBG powder (6 g) was suspended in dichloromethane with the aid of ultrasound. Simultaneously, polycaprolactone (PCL) granules (4 g) were dissolved in the same quantity of dichloromethane by magnetic stirring at room temperature. The dichloromethane containing the MBG powder was then added to the completely dissolved PCL–dichloromethane solution. This mixture was allowed to evaporate by continuous magnetic stirring at room temperature until it had formed a paste with the right consistency for injection.

2.2.2. Rapid prototyping: 3-D printing layer by layer

MBG_Scs were prepared by introducing paste into a polyethylene injection cartridge that was fixed in an EnvisionTEC GmbH 3-D Bioplotter™ printing device. The injection parameters were determined using the computer program PRIMCAM version 2.98, which directs the injector robot. The speed of the tip was set at 320 mm min^{-1} in the horizontal plane and 50 mm min^{-1} in the vertical plane. The orifice of the polyethylene conical tip used was 0.58 mm. The dimensions of the MBG_Scs were 8 mm × 8 mm × 4 mm, and the scaffolds consisted of 10 layers. The MBG_Scs obtained were dried in an oven at 70 °C for 2 h to evaporate the dichloromethane and then calcined at 500 °C for 3 h to remove the PCL.

2.3. Characterization of scaffolds

Powder X-ray diffraction (XRD) experiments were performed with a Philips X'Pert diffractometer equipped with Cu K_α radiation (wavelength = 1.5418 Å). XRD patterns were collected in the 2θ range between 0.6° and 8°, with a step size of 0.02° and a counting time of 5 s per step.

Nitrogen adsorption–desorption at 77.35 K was used to determine the textural properties using a Micromeritics ASAP 2020 porosimeter. Before adsorption measurement, the MBG_Scs were degassed under a vacuum for 24 h at 120 °C. The surface area was obtained by applying the Brunauer–Emmett–Teller (BET) method. The pore size distribution was determined by the Barret–Joyner–Halenda method from the adsorption branch of the isotherm.

The macroporosity of the MBG_Scs was examined by means of environmental scanning electron microscopy (FEI Quanta 200, Fei Company, The Netherlands).

Thermogravimetry (TG) and differential thermal analyses (DTA) were carried out in a Pyris Diamond TG/DTA thermal analyzer using an air flow of 200 ml min^{-1} and heating from 35 to 1000 °C at 5 °C min^{-1}.

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