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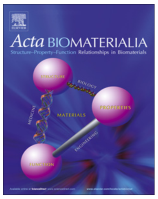

Full length article

Biocompatibility and bioactivity of porous polymer-derived Ca-Mg silicate ceramics

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ABSTRACT

Magnesium is a trace element in the human body, known to have important effects on cell differentiation and the mineralisation of calcified tissues. This study aimed to synthesise highly porous Ca-Mg silicate foamed scaffolds from preceramic polymers, with analysis of their biological response. Akermanite (Ak) and wollastonite-diopside (WD) ceramic foams were obtained from the pyrolysis of a liquid silicone mixed with reactive fillers. The porous structure was obtained by controlled water release from selected fillers (magnesium hydroxide and borax) at 350 °C. The homogeneous distribution of open pores, with interconnects of modal diameters of 160–180 μm was obtained and maintained after firing at 1100 °C. Foams, with porosity exceeding 80%, exhibited compressive strength values of 1–2 MPa. *In vitro* studies were conducted by immersion in SBF for 21 days, showing suitable dissolution rates, pH and ionic concentrations. Cytotoxicity analysis performed in accordance with ISO10993-5 and ISO10993-12 standards confirmed excellent biocompatibility of both Ak and WD foams. In addition, MC3T3-E1 cells cultured on the Mg-containing scaffolds demonstrated enhanced osteogenic differentiation and the expression of osteogenic markers including Collagen Type I, Osteopontin and Osteocalcin, in comparison to Mg-free counterparts. The results suggest that the addition of magnesium can further enhance the bioactivity and the potential for bone regeneration applications of Ca-silicate materials.

Statements of Significance

Here, we show that the incorporation of Mg in Ca-silicates plays a significant role in the enhancement of the osteogenic differentiation and matrix formation of MC3T3-E1 cells, cultured on polymer-derived highly porous scaffolds. Reduced degradation rates and improved mechanical properties are also observed, compared to Mg-free counterparts, suggesting the great potential of Ca-Mg silicates as bone tissue engineering materials. Excellent biocompatibility of the new materials, in accordance to the ISO10993-5 and ISO10993-12 standard guidelines, confirms the preceramic polymer route as an efficient synthesis methodology for bone scaffolds. The use of hydrated fillers as porogens is an additional novelty feature presented in the manuscript.

1. Introduction

In tissue engineering and regenerative medicine, synthetic or natural porous scaffolds can be implanted into a defect to guide and stimulate the formation of bone *in situ* [1]. The scaffolds are used as templates for tissue growth and should be resorbed by dissolving at controlled rates as the new tissue forms [2,3]. The scaffolds should ideally not only provide a passive structural support for cells with well-defined mechanical and physical properties (in terms of compressive strength, porosity, pores interconnectivity), but they should also stimulate osteoblastic cell proliferation and differentiation [4].

Significant attention has been given to Ca-silicates, due to their excellent bioactivity and biodegradability [5]. In fact, previous studies have shown that, at a certain concentration range, Ca and Si ions stimulate cell proliferation [6,7]. In detail, soluble silica

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has a well-established role in the bioactivity mechanism proposed by Hench [8], in osteoblast proliferation [9–11] and bone mineralisation [12,13], while Ca is known to act in regulating osteogenesis [14,15], promoting osteoblast proliferation and differentiation [16,17].

Wollastonite ($CaSiO_3$) has been widely investigated, due to its ability to bond with living bone through the formation of an apatite interface layer [18,19]. Wu et al. even incorporated wollastonite powders in poly(lactide-co-glycolide) microspheres, used for drug delivery in regenerative medicine, to successfully modulate their bioactivity for bone repair application [20]. However, porous wollastonite has low mechanical strength and high degradation rates, which may not support new tissue growth or lead to high pH values, having detrimental effects on cells [21]. Wu et al. reported that a wollastonite scaffold of 82% porosity, produced by polymer sponge method, exhibited a compressive strength of only 300 kPa [21].

Therefore, ion modifications of Ca-silicates have been proposed with the aim of tailoring both mechanical and biomedical properties. The CaO-MgO-SiO_2 system has received growing interest in the field of bioactive materials. Zhai et al. have recently reported that this family of bioceramics possess a unique dual osteogenic/angiogenic stimulatory ability [22]. Mg was found to play an important role in osteoblast adhesion [23], differentiation and proliferation [24] and on the mineralisation of bone [25,26]. Further effects of the incorporation of Mg, specifically in Ca-silicates included reduced degradation rate and improved mechanical properties. These positive consequences might be due to the higher complexity of the crystal structure after Mg incorporation: in particular, Mg-O bonds are stronger than Ca-O bonds [27,28]. Ca-Mg silicates like akermanite ($Ca_2MgSi_2O_7$) and diopside ($CaMgSi_2O_6$) are known to possess good bioactivity, slower degradation rate than wollastonite ($CaSiO_3$) and great potential as bone tissue engineering materials [29,30].

Herein, two Ca-Mg-rich ceramics were made: one had akermanite as the only crystal phase, the other was diopside coupled with wollastonite. The novelty here is in the synthesis process, involving preceramic polymers, and in the shaping method, exploiting hydrated fillers as porogens. We have previously developed wollastonite coupled with an external layer of carbonate hydroxyapatite (HCA) [31], which has improved mechanical behaviour and promising osteogenic potential. The methodology applied the pre-ceramic polymer route, that uses the thermal decomposition of the precursors and the reactions between the ceramics left behind [32,33]. The technique uses low-cost, commonly available polymeric precursors (silicone resin), filled with powdered metal oxide precursors. The advantages of polymer-derived ceramics are: they can be easily shaped into highly porous bodies when the mixture is still in the polymeric form (<350 °C) and subsequently ceramised; the firing is generally completed at low temperatures (<1200 °C); the phase purity of the ceramised products is usually high.

The present study explored the cellular response of MC3T3 pre-osteoblast cells to the two Ca-Mg-rich ceramics. Cytotoxicity was tested in accordance to the ISO10993-5 and ISO10993-12 standard guidelines. Cell attachment, viability and osteogenic differentiation were also assessed to investigate the role of Mg.

2. Experimental

2.1. Starting materials

A commercially available silicone resin (H62C, Wacker Chemie GmbH, Munich, Germany), which is liquid at room temperature, was chosen as a silica precursor, which provides a silica yield of 58 wt% when thermally treated [34]. CaO and MgO precursors consisted of $CaCO_3$ (Industrie Bitossi, Vinci, Italy) and $Mg(OH)_2$ (Industrie Bitossi, Vinci, Italy) in the form of microparticles (<10 μm). Borax (sodium borate decahydrate microparticles, $Na_2B_4O_7 \cdot 10H_2O$, Normapur Prolabo, France) was used as an additional foaming agent. All the other chemicals and reagents were purchased from Invitrogen/Life Technologies Ltd. (Paisley, UK) and Sigma-Aldrich (Gillingham, UK).

The molar balance among the most important constituents (silicone:CaO precursor:MgO precursor) followed the stoichiometric SiO_2:CaO:MgO molar proportions of akermanite (i.e. CaO:MgO:SiO_2 = 2:1:2), or of a 50 mol% wollastonite:50 mol% diopside mixture (i.e. CaO:MgO:SiO_2 = 2:1:3). Samples will be referred to as "Ak" and "WD" respectively.

2.2. Foam preparation

H62C was first dissolved in isopropanol (10 g in 13 ml) and then mixed with micro-sized fillers, including sodium borate in the as-received hydrated form (the quantity of salt was 3 wt% of the theoretical ceramic yield of the other components). Stable and homogeneous dispersions in isopropanol were obtained by magnetic stirring and ultrasonication, then were left to dry overnight at 60 °C. After first drying, the mixtures were in the form of thick pastes, later manually transferred into cylindrical Al moulds and then subjected to a heat treatment for foaming at 350 °C in air for 30 min. Cylindrical samples, 10 mm in diameter and 10 mm in height, were obtained from the foams. After polishing of the top surfaces, by means of abrasive paper, the samples were thermally treated at 1100 °C in air for 1 h at a heating rate of 2 °C min^{-1}.

2.3. Characterisation

The bulk density (ρ_b) of the foam, the quotient of the dry mass of a specimen divided by the exterior volume, including pores, was determined using a calliper and a digital balance. The skeletal density (ρ_s), the ratio of mass of discrete pieces of solid material to the sum of the volumes of the solid material in the pieces and closed or blind pores within the piece, was measured on foams, using a He gas pycnometer (Micromeritics AccuPyc 1330, Norcross, GA). Percentage porosity (%P) was then calculated using Eq. (1), where ρ_b was the bulk density and ρ_s the skeletal density:

$$\%P = 1 - (\rho_b/\rho_s). \tag{1}$$

Mercury intrusion porosimetry (PoreMaster 33, Quantachrome UK) was used to determine the pore interconnect size distribution. As the technique was destructive, it cannot be used to follow the change in pore sizes of the same scaffold as a function of sintering temperature. Optical stereomicroscopy (AxioCam ERc 5s Microscope Camera, Carl Zeiss Microscopy, Thornwood, New York, US) on foams and scanning electron microscopy (JSM 6010 L, JEOL USA; LEO GEMINI 1525 FESEM, LEO electron microscopy, Thornwood, New York, USA) on chromium-coated specimens were used to examine the morphological and textural features of the samples.

The phase identification was performed, on powdered samples obtained by manual grinding with mortar and pestle to below 100 μm, by X-ray diffraction (XRD; Bruker AXS D8 Advance, Bruker, Germany; CuKα radiation, 0.15418 nm, 40 kV–40 mA, 2θ = 10°–70°, step size = 0.05°, 2 s counting time), supported by data from PDF-2 database (ICDD-International Centre for Diffraction Data, Newtown Square, PA) and Match! programme package (Crystal Impact GbR, Bonn, Germany).

The compressive strength of foams was measured at room temperature, using an Instron 1121 UTM (Instron Danvers, MA), equipped with a 10 kN load cell, with a crosshead speed of