Silica-bonded apatite scaffolds from calcite-filled preceramic polymers

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A B S T R A C T

Silica-bonded apatite ceramics were successfully fabricated starting from preceramic polymers, in the form of silicone resins, incorporating powdered calcite ($CaCO_3$). Silicone-calcite slurries were used for infiltrating sacrificial reticulated PLA scaffolds obtained by direct 3D printing. After cross-linking the mixture at room temperature, the samples were thermally treated at $350\,^\circ C$ to burn out the PLA, thus obtaining the desired scaffolds by negative replica. The samples were later ceramized at $600\,^\circ C$, avoiding the decomposition of $CaCO_3$. A further treatment was applied to convert calcite into apatite (Ap), by immersion of samples in a phosphatizing bath (aqueous solution of sodium phosphate). It was demonstrated that the same procedure could be followed when replacing commercial $CaCO_3$ with a material of biological origin, i.e. eggshell waste, resulting in a more extensive $CaCO_3$-to-Ap conversion. The formation of the desired crystalline phases was assessed by means of XRD analysis. The scaffolds were found to exhibit a porosity between 57 and 69%, considering both the voids in the reticulated structure and the micro-sized porosity of the struts, and an excellent compressive strength, in the order of 13–16 MPa.

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1. Introduction

Natural bone, like any other connective tissue, consists of an extracellular matrix (ECM) and cells. Among the various specific types of cells, osteoblasts, osteoclasts, stomal fibroblasts, adipocytes, endothelial and hematopoietic cells are present. The ECM is here specifically mineralized, so it is made of 35% organic and 65% minerals. The organic matrix is proteic, being composed of mainly collagen-1 and fibronectin. Concerning the inorganic fraction, the most abundant mineral is hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), a calcium phosphate that is found within and between the length of collagen fibers, where it is needed to resist bending and compression [1]. Hence, hydroxyapatite ceramics have been successfully implemented in clinical applications for bone repairing, due to the excellent osteoconductive properties [2].

Even if the inorganic phase of bone has been idealized as hydroxyapatite (HAp), studies on biological apatites demonstrated that the bone mineral contains 4–8% of carbonate groups in its apatitic structure [3], thus the inorganic component of bone should be referred to as carbonate apatite (HCA), in which CO_3^{2-} ion sub-

stitutes PO_4^{3-} or OH^- ion. It has also been demonstrated that there is a strong relationship between carbonate substitution in apatitic structures and solubility of apatite in weak acid conditions (the environment produced by osteoclasts to dissolve the bone minerals has a pH of about 3–5) [4]. Specifically, the solubility of apatite increases with the increase in carbonate content of apatite, so while HAp cannot be totally resorbed by osteoclasts, HCA has a high solubility and it can be completely replaced by new natural bone, through the remodelling cycle of the bone tissue [5]. Therefore, HCA would be a more preferable choice as a bone substitute biomaterial than pure HAp.

The issue concerning the synthesis of dense HCA deals with its lack of thermal stability at the high temperatures that are required for the sintering process, due to the decomposition of the material and the subsequent release of CO_2 [6,7]. However, a dense structure is not the best candidate for replacing bone, whereas a HCA scaffold would better mimic the typical morphology of cancellous bone when shaped in an interconnected porous structure, thus promoting rapid cell penetration and revascularization.

Sunouchi et al. [5] reported a novel method to obtain solid and hollow HCA microspheres, which could be packed giving a 3-D interconnected porous structure. Calcite ($CaCO_3$), calcium hydroxide ($Ca(OH)_2$) and sodium chloride (NaCl) cores were covered with a further layer of $Ca(OH)_2$ and then treated with CO_2 for two weeks to obtain the conversion to $CaCO_3$ by carbonation. The obtained

spheres were later phosphatised in a Na_2HPO_4 solution for two weeks, successfully converting $CaCO_3$ into HCA by dissolution-precipitation reactions. Maruta et al. [8] applied the same approach to convert $CaCO_3$ foams into HCA foams. In this case, cellular materials were obtained by negative replica, using polyurethane (PU) sponges as templates and dipping them in a slurry consisting of $Ca(OH)_2$ dispersed in distilled water. After drying the slurry and burning out the PU substrate, the foams were converted to $CaCO_3$ (again by carbonation) and later phosphatised (by immersion in the same Na_2HPO_4 solution).

We already followed a similar approach in a previous paper to convert calcite into apatite [9], demonstrating the possibility of fabricating wollastonite-apatite foams from direct foaming of a slurry consisting in a liquid preceramic polymer mixed with fillers. After synthesising calcite foams, they were first phosphatised to obtain a partial $CaCO_3$-to-Ap conversion and later ceramized at 1100 °C to allow the reaction between the remaining calcite and silica left from the silicone-to-ceramic conversion, thus obtaining wollastonite-apatite composite foams.

In the present study, an easy method is proposed to fabricate silica-bonded apatite porous structure by negative replica, starting from PLA 3D printed sacrificial templates. Here, the goal was to obtain a significant conversion of calcite into apatite. Liquid preceramic polymers mixed with powdered $CaCO_3$ fillers were used as a slurry to infiltrate PLA foams and they were cross-linked at room temperature prior to burning out the PLA. After ceramization at 600 °C, particularly low compared to the temperatures adopted for other silicone-derived ceramics [10], the ceramic scaffolds were phosphatised by immersion in a Na_2HPO_4 bath, following the same procedure as Sunouchi et al. [5] and Maruta et al. [8]. The $CaCO_3$-to-Ap conversion was even more successful when $CaCO_3$ was replaced with a biological one, derived from eggshell waste, due to its natural impurities facilitating the interdiffusion phenomena. The silica binding phase is reputed to be favourable, owing to the well-known positive effects of Si ions on bone formation [11–14].

2. Experimental

2.1. Materials and methods

Two commercial liquid silicones, RTV90700 (Siliconi Padova, Italy) and RTV960 (Angeloni, Italy) constituted the reference materials for the silica binder. The two bi-component polymers feature a silica yield of 72.5% and 58 wt%, respectively, and can cross-link at room temperature, by a polyaddition mechanism, when mixed with their specific curing agents. RTV90700 and RTV960 were used to obtain 67 and 33 wt% of the total silica content, respectively, so that the polymers were used in the weight ratio of RTV90700/RTV960 = 1.6. The silicone mixture was added with calcite powders, for a nominal $CaCO_3/SiO_2$ weight ratio of 70/30. Calcite ($CaCO_3$) was used in two variants of micro-sized powders: (i) commercial calcite (<10 μm, Industrie Bitossi, Vinci, Italy – later referred to as "BIT"); (ii) calcite from eggshell waste, after a heating cycle up to 450 °C, for 4 h, with a heating rate of 5 °C/min (<17 μm, later referred to as "ESW"). The egg shells were obtained from Lecoque Eggs (Tongeren, Belgium), one of the market leaders in the European egg products sector. The received ESW were already dried and crushed down to 3 mm. To reduce the size further the as received ESW was further ball-milled (2 × 10 min at 300 rpm with 30 mm agate milling balls followed by 2 × 10 min with 2 mm zirconia milling balls) in a dry state down to a d_{90} of 17 μm. The resulting BET of the ESW powder is 4–6 m^2/g. The two calcite powders were subjected to energy dispersive X-ray fluorescence (XRF) analysis (Spectro X-LAB 2000, Spectro Analytical Instruments GmbH, Kleve, Germany).

RTV90700 and RTV960 were homogeneously mixed by mechanical stirring, then the $CaCO_3$ powders were incorporated. Only for the filler derived from eggshell waste, a small amount of solvent (isopropyl alcohol) was added (1 ml for 10 g of final ceramic), because the viscosity of the slurry tended to increase rapidly as the powders were incorporated. At last, the curing agents for the two polymers were added and the resulting mixtures were further subjected to mechanical stirring, in order to obtain homogeneous pastes.

Before the occurrence of cross-linking reactions, silicone/calcite slurries were used to infiltrate, by immersion, 3D-printed reticulated polylactic acid (PLA) scaffolds, in turn obtained by means of a Power WASP orienting extruder (Massa Lombarda, Italy). The PLA scaffolds were placed at the bottom of a plastic container and left immersed in the slurries for 12 h, until the silicone/calcite pastes became rubbery. At this stage, the excess paste was removed from the PLA scaffold by means of a cutter.

The infiltrated PLA scaffolds were thermally treated at 350 °C, with a heating rate of 0.5 °C/min, for 1 h. This step led to the decomposition of PLA, obtaining reticulated structures (replicas) made of cross-linked silicones and $CaCO_3$. The replicas were later subjected to a ceramization treatment at 600 °C, with a heating rate of 2 °C/min, for 2 h, in order to transform the silicones into amorphous silica, while maintaining $CaCO_3$ unreacted.

After ceramization, the scaffolds were subjected to a phosphatization treatment by immersion in a 1 mol/L Na_2HPO_4 solution at 60 °C for 14 days, resulting in silica-bonded apatite ceramics.

2.2. Samples characterization

Micro-structural characterizations were performed by optical stereomicroscopy (AxioCam ERc 5s Microscope Camera, Carl Zeiss Microscopy, Thornwood, New York, US), scanning electron microscopy (FEI Quanta 200 ESEM, Eindhoven, The Netherlands) equipped with EDS and X-ray diffraction (XRD; Bruker AXS D8 Advance, Bruker, Germany – CuKα radiation, 0.15418 nm, 40 kV–40 mA, 2θ = 15–70°, step size = 0.05°, 2 s counting time). The Match! software package (Crystal Impact GbR, Bonn, Germany) was used for phase identification, supported by data from PDF-2 database (ICDD-International Centre for Diffraction Data, Newtown Square, PA, USA) and Crystallography Open Database (COD) database [15].

The bulk density (ρ_b) of the foams was determined using a caliper and a digital balance. The skeletal density (ρ_s) was measured on foams, using a He gas pycnometer (Micromeritics AccuPyc 1330, Norcross, GA). The percentage of porosity was then calculated using the following equation:

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

Samples were analyzed by attenuated reflectance Fourier transform infrared spectroscopy (ATR-FTIR, Thermo Scientiic Nicolet iS10) at room temperature, to monitor the growth of the hydroxy-carbonate apatite layer, using a wavenumber resolution of 4 cm^{-1} for 32 scans from 4000 to 600 cm^{-1}. Selected scaffold structures were subjected to mechanical characterization in compression mode at room temperature, using an Instron 1121 UTM (Instron Danvers, MA, USA) with a cross-head speed of 1 mm/min, on samples in the as-ceramized form and after phosphatization. Each data point is presented as the mean value of five to ten samples.

2.3. Results and discussion

The results from XRF analysis, shown in Table 1, demonstrate that both types of calcite contained some impurities. The overall amount of metal oxides ranges between 59 (BIT) and 56 (ESW) wt%;