Original Article

Direct ink writing of silica-carbon-calcite composite scaffolds from a silicone resin and fillers

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A B S T R A C T

Calcite-based composite scaffolds have been successfully 3D-printed by direct ink writing, starting from a paste comprising a silicone polymer and calcite ($CaCO_3$) powders. The firing in nitrogen, at 600 °C, after preliminary cross-linking step at 350 °C, determined the transformation of the polymer matrix into a silica-carbon nano-composite, embedding unreacted calcite particles. Compared to previously developed silica-calcite scaffolds, obtained after firing in air, the new composites exhibited a significant strength improvement (up to ~10 MPa, for a total open porosity of 56%). The new formulation did not compromise the in vitro bioactivity and the biocompatibility of the scaffolds, as shown by dissolution studies in SBF and preliminary cell culture tests, with human fibroblasts. Due to the simplicity of the processing and the outstanding mechanical performances, the developed scaffolds are promising candidates for bone tissue engineering applications.

1. Introduction

Calcium carbonate ($CaCO_3$), in its polymorphic variants (calcite, aragonite, vaterite), is well known for its high biocompatibility and bioactivity [1–6]. These features are appreciated in the material from both synthetic and natural (e.g. deriving from shells) origin [7]. In particular, the bone forming ability of calcium carbonate is comparable to that of hydroxyapatite (HAp) [8], and the newly formed bone mimics very well the natural one (owing to the inclusion of carbonate groups in the apatite crystal structure, more properly termed as 'carbonated apatite', or HCA) [9].

Any modern biomaterial for bone tissue applications, however, must obey to strict morphological requirements. A three-dimensional network structure, with interconnected macro-pores, is needed for cell ingrowth and vascularization to be effective [10]. Among calcium carbonate-based materials, marine corals (mostly composed of aragonite) are known to naturally fulfil the requirements, but their use as bone graft substitutes, after successful experiments dating back to the 1980s [11,12], is no longer accepted, mainly for risks of viral or bacterial contamination. Synthetic $CaCO_3$-based porous material are therefore highly attractive, although conditioned by several processing problems. As an example, the thermal decomposition of $CaCO_3$ at a relatively low temperature represents a strong limitation to the sintering [13]. This limitation may be overcome by controlling the sintering atmosphere (carbon dioxide, instead of air [3]), by using a sintering aid (such as biodegradable phosphate glass [14]), or adopting a quite complex procedure, implying the use of $Ca(OH)_2$ slurries, deposited one polyurethane (PU) templates and transformed into $CaCO_3$ by carbonatation, after PU burn-out [15].

The degradation rate of $CaCO_3$ may result excessively rapid. As an example, Zhong et al. showed that an optimum degradation could be found using composite $CaCO_3$-hydroxiapatite microspheres (prepared by hydrothermal method), instead of pure $CaCO_3$ microspheres [16].

The 'composite approach' was at the basis of recent experiences, concerning the use of preceramic polymers, namely silicone resins, as matrices for $CaCO_3$ [17,18]. The particular matrices enabled the transformation into silica-calcite composites, by thermo-oxidative transformation of the polymers, at moderate temperature (600 °C), i.e. without any decomposition of $CaCO_3$. In addition, silicones enabled the easy manufacturing of highly porous scaffolds, by application of both indirect and direct additive manufacturing techniques. More precisely, reticulated three-dimensional structures were obtained by infiltration

of silicone-$CaCO_3$ slurries in 3D-printed sacrificial polymeric templates [17] and by direct ink writing of silicone-$CaCO_3$ pastes [18], respectively. The potential of the developed scaffolds can be envisaged from the successful conversion into silica-hydroxyapatite scaffolds [17], as well as from tests with bone marrow stromal cells, found to be alive, well anchored and spread on the samples surface [18].

The present investigation was conceived in order to further extend the approach, with a particular attention to the inclusion of a carbon phase. Bioceramics containing carbon, in the form of graphene or carbon nanotubes, were recently proposed, with positive results on mechanical properties and cytocompatibility [19–21]. The use of silicone polymers actually brought the possibility to form a carbon phase directly 'in situ', owing to the control of the ceramic conversion operated by the firing atmosphere: in fact, when heated in nitrogen, silicones do not transform simply into amorphous silica, but into amorphous silicon oxycarbide (SiOC). SiOC may be considered as an "over-bridged" silica glass, including some Si-C bonds in the siloxanic network, mixed with turbostratic carbon nano-sheets, evolving into silica/SiC/C composite for treatments above 1200 °C [22]. We will show that, owing to the particular conditions adopted, Si-C bonds did not form, with all carbon as separate phase; in any case, C-containing composites exhibited a very remarkable strength improvement compared to C-free counterparts, with no degradation of biocompatibility.

2. Experimental procedure

2.1. Manufacturing of scaffolds

A commercial polymethylsiloxane, SILRES® MK (Wacker-Chemie GmbH, München, Germany), featuring a silica yield of 84 wt% after thermal decomposition in both air and nitrogen [18], was used for the fabrication of a "preceramic ink", combined with nano-sized silica (fumed silica, Aerosil R106, Evonik Germany). Fumed silica, in the weight proportion MK/fumed silica = 11/1, was adopted to adjust the rheological behavior, as previously done with several silicone/filler mixtures [18,23,24]

Fumed silica powders were wet mixed with MK in isopropanol (8 ml for 16 g of silica precursors) by means of a ball mill (60 min at 100 rpm, Pulverisette 7 planetary planetary ball mill, Fritsch, Idar-Oberstein, Germany). The polymer was added with $CaCO_3$ micro-sized powders (< 10 μm, Industrie Bitossi, Italy) - in the weight proportion MK/$CaCO_3$ = 1/1.7 - and mixed again (4 h at 400 rpm), to obtain a perfectly homogenous suspension with very fine fillers and no trace of powder aggregates.

Silicone-based pastes were extruded by means of a PowerWASP orienting extruder (Massa Lombarda, Italy), specifically equipped with a syringe ending with a conical nozzle (diameter of 0.81 mm, Nordson EFD, Westlake, Ohio). Filaments were extruded on a glass substrate immersed in vegetal oil "Sunflower", thus preventing the premature drying of the solvent that could modify the viscosity of the ink.

Following a CAD file, scaffolds were in the form of prisms with dimensions 20 mm x 20 mm x 7.2 mm, as resulting from the overlapping of cylindrical rods, periodically arranged along x and y-axes. The rods were in a stacking on the x–y plane with the distance between the longitudinal axes of adjacent rods was of 1.6 mm. The spacing between adjacent rods along the z axis was set at 600 μm.

After printing, the scaffolds were left in oil to dry overnight at room temperature. Then, the scaffolds were simply removed from the oil bath and left over paper tissue to take out the excess oil. After oil removal, printed scaffolds were cross-linked at 350 °C, with a heating rate of 0.5 °C/min and dwelling time of 1 h, prior to ceramization at 600 °C in flowing nitrogen (same heating rate and dwelling time as the cross-linking treatment), in a tubular furnace (Carbolite CTF 12, Hope, UK). After 1 h at 600 °C, the ceramized samples were subjected to natural cooling inside the furnace.

2.2. Microstructural and mechanical characterization

Micro-structural characterizations were performed by means optical stereomicroscopy (AxioCam ERc 5 s Microscope Camera, Carl Zeiss Microscopy, Thornwood, New York, US) and scanning electron microscopy (FEI Quanta 200 ESEM, Eindhoven, The Netherlands) equipped with EDS. The mineralogical analysis was performed by means of X-ray diffraction (XRD; Bruker AXS D8 Advance, Bruker, Germany - CuKα radiation, 0.15418 nm, 40 kV-40 mA, 2θ = 20–70 °, step size = 0.05°, 2 s counting time), on powdered samples. The powders comprised silicone-$CaCO_3$ mixture pyrolysed in nitrogen (CN samples), as well as fired in air (CA samples, for comparison purposes; CA samples were identical to those already presented [18]). A semi-automated phase identification was provided by the Match! software package (Crystal Impact GbR, Bonn, Germany), in turn operating on data from PDF-2 database (ICDD-International Centre for Diffraction Data, Newtown Square, PA, USA).

Photoemission spectra were collected using a custom made UHV system operating at a base pressure of 10^{-9} mbar and equipped with an EA125 Electron analyzer (Scienta Omicron, Taunusstein, Germany) and a dual anode Mg-Al 400 W X-ray source (DAR 400, Scienta Omicron). All measurements were taken at room temperature using a pass energy of 50 eV for survey and 20 eV for high resolution spectra, on powdered samples. The powders were suspended in milliQ water, thoroughly sonicated at RT for 30′; then an amount of 200 μl of the resulting suspension was drop-casted on polished electrochemically pure copper substrates. In this way, quite homogeneous thin films were obtained. Such samples were then introduced into the UHV system, degassed overnight and annealed at about 110 °C in ultrahigh vacuum, just before the acquisition of the XPS measurements, in order to remove debris and adsorbed water. All XPS measurements were acquired at room temperature suing a Mg Kα emission line. Due to the insulating nature of the samples, charging was observed during the measurements, therefore the energy scale of the spectra has been corrected using the Ag $3d_{5/2}$ photoemission line coming from a silver bulk piece in electrical contact with the samples. The procedure was repeated for a sample fired in nitrogen after HF etching (5 g of ceramic powders immersed in 20 cc of HF 20 vol% for 4 weeks).

The geometrical density of the cellular samples was determined using a caliper and a digital balance. The apparent density and the true density were determined by means of He gas pycnometer (Micromeritics AccuPyc 1330, Norcross, GA), operating on whole scaffolds or on powdered samples, respectively.

The compressive strength, at room temperature, was measured using an Instron 1121 UTM (Instron Danvers, MA, USA), with a cross-head speed of 1 mm/min. Each data point represents the mean value of ten samples.

2.3. Assessment of the in vitro bioactivity

Before the biological evaluation (see the following section), the bioactivity of the produced scaffolds was preliminary investigated in vitro in an acellular Simulated Body Fluid solution (SBF), according to the procedure originally defined by Kokubo e Takadama [25]. The samples, with weight of 100 mg, were immersed in polyethylene flasks containing 25 ml of SBF and stored at 37 °C in an incubator (MPM Instruments s.r.l., Bernareggio, Milano, Italy). The solution was refreshed every about 2 days and the pH of the SBF was measured. At fixed times of 1, 3, 7 and 14 days, the scaffolds were extracted from the medium, washed in bi-distilled water and dried at room temperature for 24 h before further characterization. The surface of samples was observed in a SEM (ESEM Quanta 200, FEI Co., Eindhoven, The Netherlands) to evaluate microstructural changes that occurred during the immersions and, in particular, the possible precipitation of hydroxycarbonate apatite (HCA). Local chemical analyses were performed by X-ray energy dispersion spectroscopy, EDS (Inca, Oxford Instruments, UK). The