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Sintering effects of bioactive glass incorporation in tricalcium phosphate scaffolds

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Abstract

The influence of using bioactive glass as a sintering aid in the production of tricalcium phosphate (TCP) scaffolds was investigated. The scaffolds were fabricated by sponge replication followed by sintering in a range of temperatures from 1150 to 1300°C. Morphological investigations by SEM and micro-computed tomography showed that the scaffolds exhibited a three-dimensional trabecular architecture mimicking that of cancellous bone, with high porosity (about 80 vol.%) and highly-interconnected macropores with mean pore size of 314 μm. Apart from playing a role in improving the mechanical properties of the scaffolds, glass was shown to enhance the stability of β-TCP by increasing the β→α phase transition temperature.

Keywords: Bioceramics; Porous materials; Bioglass; Scaffold; Calcium phosphate.

1. Introduction

Calcium phosphate bioceramics have been clinically used for decades as bone substitutes due to their chemical similarity to the mineral phase of bone [1]. Hydroxyapatite and β-tricalcium phosphate (β-TCP) are the most important members of this family of compounds [2]. While hydroxyapatite is osteoconductive and non-resorbable – unless in the form of nano-powders –, β-TCP exhibits a mild resorbability in the body [3]. β-TCP ceramics are commonly produced by high-temperature sintering but, if thermally-treated above 1150°C, the β phase irreversibly changes to α phase that is then retained at room temperature [4]. α-TCP is quickly resorbable and, thus, contraindicated in those applications requiring mechanical support over time, such as defect filling in load-bearing bones [5].

The significant limitation of β-TCP products is their low mechanical strength, since their sintering temperature is limited by the β→α transition. This drawback is emphasized when the goal is the fabrication of three-dimensional (3D) porous bioceramic scaffolds [6]. Increasing the β→α transition temperature could overcome this limitation, enhancing the high-temperature stability of β-TCP.

In the present work, we investigate this aspect and suggest the use of bioactive glass as a sintering aid to produce β-TCP scaffolds; although a similar approach was applied in the past to the sintering of hydroxyapatite [7,8], to the best of the authors' knowledge it was not proposed for β-TCP so far.

2. Materials and methods

2.1 Preparation

Starting materials include TCP particles (purity >98%, Intrauma SpA, Italy) and melt-derived CEL2 bioactive glass ($45SiO_2$-$3P_2O_5$-26CaO-7MgO-$15Na_2O$-$4K_2O$ mol.%). Details of production and applications of CEL2 in bone regeneration were reported in previous works [9,10]. Before being used, TCP and glass particles were ball-milled (Pulverisette 6, Fritsch, Idar-Oberstein, Germany) and then sifted (stainless steel sieve, Giuliani Technologies Srl, Torino, Italy) to obtain powders with size below 32 µm.

A sacrificial 45-ppi polyurethane sponge was used to produce porous scaffolds by the foam replica method. Sponge cuboids (side ~10 mm) were dipped into a water-based ceramic slurry (25% solid load, 69% distilled water, 6% poly(vinyl alcohol) used as a binder (wt.%)). The solid load comprised either 100% TCP particles or a mix of TCP and glass particles; the glass was added at 3 wt.%, as previously suggested by Knowles et al. (2-4 wt.% [7]) for preparing glass-reinforced hydroxyapatite. After being extracted from the slurry, the samples were compressed to squeeze the suspension out of the pores [11]. The samples were air-dried overnight at room temperature and then thermally treated in the range of 1150 to 1300°C for 12 h (heating rate 10°C/min) in order to burn off the polymeric sponge and sinter the inorganic particles, thus obtaining a positive replica of the sacrificial template.

2.2 Characterization

Crystalline phases were investigated by using X-ray diffraction (XRD) method on powdered scaffolds (X'Pert diffractometer with source voltage 40 kV and current 30 mA, Bragg-Brentano camera geometry, Cu Kα radiation, wavelength λ = 15.405 nm, scan range 2θ within 10-50°, 2θ step 0.02°, fixed counting time 1 s per step). Phase identification was performed by using X'Pert HighScore software equipped with PCPDFWIN database. The mass ratio of the phases was estimated by the standard reference intensity ratio (RIR) method.

The scaffolds were sputter-coated with chromium and their morphology and 3D porous architecture were investigated by field-emission scanning electron microscopy (FESEM, Supra^{TM} 40 Zeiss, Germany; accelerating voltage = 15 kV) equipped with X-ray energy dispersive spectroscopy (EDS).

Scaffold shrinkage was determined by volumetric measurements before and after sintering. Total porosity of the scaffolds was assessed by post-sintering mass-volume measurements.

Non-destructive investigations were performed by micro-computed tomography (micro-CT; SkyScan 1172, Bruker, Belgium) on selected samples in order to obtain a quantitative assessment of the key microstructural parameters. Sample volume was reconstructed from raw micro-CT data through the standard filtered back-projection algorithm [12].

The compressive strength σ_c (MPa) was assessed through crushing tests on 8-mm porous cuboids by using a Syntech 10/D apparatus (50 N cell load, cross-head speed 1 mm/min). Specifically, the strength was calculated as $\sigma_c = F/A$, where F (N) was the maximum load registered during the test and A (mm^2) the resistant cross-sectional area.

Results of shrinkage, porosity and compressive strength are reported as mean ± standard deviation calculated on five specimens.

3. Results and discussion

XRD analysis reported in Figure 1a revealed that the starting ceramic powders were composed of pure β-TCP (whitlockite, PDF code: 00-009-0169) with traces of hydroxyapatite (PDF code: 01-082-1998). If no additives are used during scaffold production, β→α transition occurs during sintering at 1150°C, as confirmed by the presence of diffraction peaks of α-TCP (PDF code: 00-029-0359, monoclinic crystal system, space group P21/a) in Figure 1b; this is in accordance with previous literature [5]. In this glass-free TCP sample, the mass ratio of α-TCP to β-TCP is around 3.2. On the contrary, if 3 wt.% of glass is added to the starting β-TCP powder during scaffold fabrication, β→α transition does not occur at 1150°C and β-TCP remains the stable phase at room temperature (Figure 1c). The ability of glass to increase β-phase stability could be associated to the presence of MgO in the glass composition and the subsequent Mg/Ca replacement in the TCP: in fact, it was reported that the transformation temperature of Mg-doped β-TCP to α polymorph increased with increasing substitution of Mg^{2+} in Ca^{2+} sites [13]. In agreement with these previous findings, it was observed a shift of peak positions in the XRD patterns: for example, the major peak of β-TCP shifted from 2θ = 31.0805° in Figure 1a to 2θ = 31.1503° in Figure 1c. This shift can be due to the emplacement of Mg^{2+} ions into the β-TCP structure and the resulting changes in lattice parameters. In this regard, qualitative phase identification suggests the presence of $(Ca_{2.86}Mg_{0.14})(PO_4)_2$ (PDF code: 01-077-0692), the peaks of which are overlapped to those of β-TCP. This β-T(Ca,Mg)P phase can be quantified only if magnesium content exceeds 14 mol.% [7], otherwise it should be considered negligible; however, even a very low amount of Mg^{2+} ions is suitable to delay the β→α transformation. In fact, the α phase starts to form at 1175°C, as demonstrated by the detection of the typical doublet at 2θ = 30.80-31.10° in Figure 1d (mass ratio of α-TCP to β-TCP around 0.6), and then becomes predominant over the β one just at 1300°C (mass ratio of α-TCP to β-TCP around 3.0) (Figure 1e).

Morphological analysis by SEM (Figure 2a) reveals that the pore-strut architecture of the polymeric template was well replicated by the scaffolds. As the sintering temperature increases, the grain size increases too, as shown by the comparison between Figure 2b and c (1175 vs 1300°C). Compositional analysis (Figure 2d) confirms the presence of the glass in the scaffold as small peaks for Si, Na and Mg, which are typical elements of the glass, were detected in the EDS spectrum.

Investigations were then focused on the glass-added scaffolds sintered at 1150°C (full stability of β-TCP). The data reported in Table 1 show that shrinkage was similar for β-TCP and glass-added β-TCP scaffolds; total porosity, which is slightly higher in the latter batch, is comparable to that of human cancellous bone (50-85 vol.% [14]).

The bone-like trabecular structure of scaffolds was further revealed by micro-CT investigation (Figure 3a). Quantitative assessment of microstructural parameters allowed determining mean pore size (314 μm), surface-to-volume ratio (a_V = 8.9 mm^{-1}) and density of interconnectivity (β_V = 55.0 mm^{-3}). As displayed in Figure 2b, most pores (90%) lie in the optimal range recommended for bone tissue engineering scaffolds (100-500 μm [14]). Interestingly, the values of a_V and β_V are about 2 and 15 times higher than those of human cancellous bone from various sources [15,16]; however, they are comparable to those of commonly-used porous sintered hydroxyapatite scaffolds (EngiPore™, Finceramica, Italy) [17]. The presence of a 3D network of interconnected macropores is key for bone tissue engineering applications in order to have paths for cells to migrate, new tissue to grow in and nutrients/waste products to flow in/out of the scaffold [14].

The compressive strength of glass-added scaffolds sintered at 1150°C was 0.14 ± 0.07 MPa: this value is in the range of cancellous bone (0.1-10 MPa [18]) – although being close to the minimum threshold of acceptance – but is 2 times higher compared to that of pure β-TCP scaffolds having analogous porosity and produced by the same method (0.064 MPa [19]). This suggests that using silicate glass as a sintering aid can play an important role in improving the mechanical performance of TCP scaffolds, in agreement with the results reported by Fielding et al. [20] who observed that silica promotes some liquid-phase sintering, which is beneficial because it results in a better densification than that of solid state sintering.

4. Conclusions

TCP scaffolds were fabricated by sponge replication and exhibited a 3D network of interconnected macropores mimicking the trabecular architecture of spongy bone. Using bioactive glass as a sintering aid allows enhancing the β-phase stability and improving the mechanical properties. When 3 wt.% of glass is added, the α phase did not form at 1150°C, which is the temperature at which the β→α transition typically occurs. If sintering temperature is increased, α-TCP starts to form and coexists with the β phase up to 1300°C. Control of phase transformation can be useful from the viewpoint of biomedical applications in order to design the resorption kinetics of the scaffold according to the clinical needs.

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Figure legends

Figure 1. XRD patterns of (a) raw TCP powders, (b) glass-free TCP scaffold sintered at 1150°C/12 h, glass-added TCP scaffolds sintered at (c) 1150°C/12 h, (d) 1175°C/12 h and (e) 1300°C/12 h. Legend: ∇ = β-TCP, ▼ = α-TCP, ● = hydroxyapatite.

Figure 2. SEM micrographs of glass-added TCP scaffolds sintered at (a) 1150°C/12 h, (b) 1175°C/12 h and (c) 1300°C/12 h; (d) compositional analysis by EDS on (b). Magnifications: (a) 300×, (b,c) 5000×.

Figure 3. Micro-CT investigations on a glass-added TCP scaffold sintered at 1150°C/12 h: (a) 3D sub-volume reconstruction and (b) pore size distribution.

Table 1. Shrinkage and porosity of scaffolds sintered at 1150°C for 12 h.

Sample	Shrinkage (%)	Porosity (vol.%)
TCP scaffold	51 ± 1	79 ± 2
Glass-added TCP scaffold	52 ± 6	83 ± 2