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Fabrication of HA/TCP scaffolds with a graded and porous structure using a camphene-based freeze-casting method

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Abstract

A room temperature camphene-based freeze-casting method was used to fabricate hydroxyapatite/tricalcium phosphate (HA/TCP) ceramic scaffolds. By varying the solid loading of the mixture and the freezing temperature, a range of structures with different pore sizes and strength characteristics were achieved. The macropore size of the HA/TCP bioceramics was in the range of 100–200 μm, 40–80 μm and less than 40 μm at solid loadings of 10, 20 and 30 vol.%, respectively. The initial level of solid loading played a primary role in the resulting porosity of the scaffolds. The porosity decreased from 72.5 to 31.4 vol.% when the solid loading was increased from 10 to 30 vol.%. This resulted in an increase in the compressive strength from 2.3 to 36.4 MPa. The temperature gradient, rather than the percentage porosity, influenced the pore size distribution. The compressive strength increased from 1.95 to 2.98 MPa when samples were prepared at 4 °C as opposed to 30 °C. The results indicated that it was possible to manufacture porous HA/TCP bioceramics, with compressive strengths comparable to cancellous bone, using the freeze-casting manufacturing technique, which could be of significant clinical interest.

Keywords: Freeze-casting; Porous; Hydroxyapatite; Bioceramics

1. Introduction

Calcium phosphate-based bioceramics have received considerable attention as bone graft substitutes because of their excellent biocompatibility, bioactivity and osteoconductive characteristics compared to other materials. One of these bioceramics is hydroxyapatite (HA), which has a similar chemical composition to the major inorganic component of the skeletal tissue of vertebrates. It has been shown that synthetic HA is totally biocompatible, nontoxic and osteoconductive [1–3]. However, although HA is bioactive, it exhibits slow osteoconduction in vivo [1]. In the last decade, attention has focused on producing highly porous HA/TCP in either block or granular form in order to promote tissue ingrowth, thus enhancing the implant-tissue attachment and improving osteoinduction [4].

It has been reported that an optimal pore size exists for successful cell infiltration and host tissue ingrowth: 5–15 μm for fibroblasts, 20–125 μm for adult mammalian skin tissues and 100–350 μm for bone tissues [5]. There is also some evidence that pore interconnectivity is as important as porosity for bone ingrowth, particularly in the early stages of bone regeneration and penetration in the scaffold [6,7].

A number of fabrication techniques have been developed over the years for manufacturing porous bioceramics. These include, amongst others, the replication of polymer foams by ceramic dip coating [8,9] or impregnation, foaming of aqueous ceramic powder suspensions [10,11], pyrolysis of preceramic precursors [12] and firing of ceramic powder compacts with pore-forming fugitive phases [13,14]. However, none of these methods can completely satisfy all the necessary requirements, namely, a controlled level of interconnected porosity combined with good mechanical properties (strength in the region of 20 MPa with a porosity >50%). An example is the foam replication

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method, which can achieve very high volumes of porosity and excellent interconnectivity levels but characteristically results in poor mechanical properties due to defects generated during the pyrolysis of the polymer foam template [9].

An alternative approach is to use the freeze-casting (or freeze-drying) process to produce porous ceramics [15]. This has proven to be an attractive manufacturing method as it allows construction of reticulated porous ceramics on a finer scale and without the polymer burnout stage. This avoids some of the inherent problems currently associated with other manufacturing methods. The process consists of freezing a ceramic slurry, which is usually aqueous based, in a mould at low temperatures, followed by demoulding and vehicle removal by sublimation to obtain a green body [15]. More recently, camphene, which has a melting point of approximately 45 °C, [15–18] has been used successfully as a freezing vehicle, allowing more flexibility in the process as it can be frozen and easily sublimed at room temperature [19–21].

The aim of this study was to explore the application of this method to the production of a highly porous calcium phosphate-based ceramic with aligned, interconnected and graded porosity for tissue engineering purposes and to determine the important variables that control the structural characteristics of the final product.

2. Materials and methods

2.1. Materials

Commercially available hydroxyapatite powder TCP130 ($Ca_{10}(PO_4)_6(OH)_2$) (Thermphos) with an average particle size of six microns was selected. The composition of the ceramic powder before, and the bulk specimens after, sintering were quantitatively assessed using X-ray diffraction (XRD). The XRD spectra were obtained between 20 and 60 (2θ) in 0.02° steps. In order to estimate the phase compositions, the Rietveld refinements were performed using a software package "Fullprof Suite" on a 2θ range between 21° and 36° using structural models for the phases identified, including HA, α-tricalcium phosphate (TCP) and β-TCP. Camphene ($C_{10}H_{16}$), 95% purity, Sigma–Aldrich Company Ltd., Gillingham, UK) was used as the freezing vehicle without any further purification. In addition, Texaphor 963 (Cognis, Southampton Hampshire, UK) was used as dispersant (density at 20 °C of 0.89–0.91 gcm^{-3} and acid value of 55–58 mg KOH g^{-1}). The dispersant concentration was 6–100 wt.% of the calcium phosphate powder for all 10, 20 and 30 vol.% of solid loadings.

2.2. Solidification study

The solidification of the slurry was monitored by preparing an HA/Camphene slurry at 60 °C with a solid loading of 2 vol.% and placing a drop into a pre-heated slide glass (both top and bottom) in order to avoid rapid freezing. A low concentration of HA was used in order for the slurry to be sufficiently translucent for analysis under the light microscope. The drop was left to freeze at room temperature, after which the structure formed was examined using optical microscopy, with particular emphasis on observing the presence of primary and secondary dendrites.

2.3. Fabrication methods for bulk samples

The first part of the manufacturing process involved the slurry preparation. This was achieved by melting the camphene at a temperature of 60 °C on a heating plate to create a clear and fluid vehicle. The dispersant concentration was 6–100 wt.% of calcium phosphate powder for all solid loadings. The HA powder was then added in quantities of 10, 20 and 30 vol.%. The slurry was stirred via the use of a motor and stirrer with a cap on the top to prevent any camphene vapour escaping. The slurry was left to stir at a constant temperature for 3 h, for all solid loadings, before pouring into metallic moulds for freezing; the moulds were 15 mm in internal diameter and 80 mm in height. Some samples were left to cool at room temperature (20 °C) for 30 min; other samples were cooled at different temperatures (4 and 30 °C) for the same length of time to study the effect of cooling rate on the solidification characteristics. After solidification, the green body was removed from the moulds and left to sublime (optimised to 20 h) at room temperature in order to remove the camphene entirely and achieve a highly porous structure. Following sublimation, sintering of the green body at 1280 °C enabled the densification of the samples and concomitant improvements in mechanical strength. The sintering regime entailed heating the samples at 35 °C h^{-1} up to 600 °C followed by 1 h of dwell time. They were then heated at 60 °C h^{-1} up to 1280 °C and held at this temperature for 4 h, before cooling down to room temperature at a rate of 120 °C h^{-1}. Prior to sintering, the ceramic powder was 100% HA. Post-sintering, XRD analysis confirmed that the composition of the ceramic body changed to 75% HA, 18% β-TCP and 7% α-TCP.

2.4. Pore structure analysis

The fabricated samples were characterised by evaluating their pore structures, including pore size and porosity, and by observing the densification of the HA/TCP walls using scanning electron microscopy (SEM; JSM 6480LV). The porosity was calculated using mass and volume measurements (using electronic balance and callipers) to determine the density of the porous samples and then comparing this to that of the fully dense ceramic, using a value from the literature of 3.16 g cc^{-1} [22], to find the percentage of "void" (15 samples per condition were examined and standard deviation calculated accordingly). Pore size was determined by measuring the average size of pores from the SEM micrographs taken at four points on each sample 3 mm from the outer wall at a 90° plane (two samples per condition and four locations per sample).

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