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Improving the compressive strength of bioceramic robocast scaffolds by polymer infiltration

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The effect of polymer infiltration on the compressive strength of β-tricalcium phosphate (TCP) scaffolds fabricated by robocasting (direct write assembly) is analyzed in this work. Porous structures consisting of a tetragonal three-dimensional mesh of interpenetrating rods were fabricated from concentrated TCP inks with suitable viscoelastic properties. Biodegradable polymers (polylactic acid (PLA) and poly(ε-caprolactone) (PCL)) were infiltrated into selected scaffolds by immersion of the structure in a polymer melt. Infiltration increased the uniaxial compressive strength of these model scaffolds by a factor of three (PCL) or six (PLA). It also considerably improved the mechanical integrity of the structures after initial cracking, with the infiltrated structure retaining a significant load-bearing capacity after fracture of the ceramic rods. The strength improvement in the infiltrated scaffolds was attributed to two different contributions: the sealing of precursor flaws in the ceramic rod surfaces and the partial transfer of stress to the polymer, as confirmed by finite element analysis. The implications of these results for the mechanical optimization of scaffolds for bone tissue engineering applications are discussed.

1. Introduction

Synthetic biodegradable scaffolds are today's most promising candidates for bone substitution and regeneration. The surgery involved in implanting synthetic bone substitutes is less invasive than for autografts (which require two surgical sites) and the former do not have the problem of available quantities of the latter. They are also free of the rejection and disease transmission risks associated with xenografts. Biodegradable scaffolds provide structural support for cell growth during regeneration of the tissue, and they are eventually resorbed, leaving only the newly formed living tissue and the fully healed lesion.

Most of the limitations associated with conventional scaffold fabrication techniques (solvent casting, fiber meshing, gas foaming, etc.) are related to a limited control over the pore structure. Fortunately, solid free-form fabrication (SFF) techniques – stereolithography, three-dimensional (3-D) printing, fused deposition modeling, robocasting, etc. – can overcome these hurdles [1,2] since they are based on a layer by layer fabrication of structures with customized and complex 3-D shapes from a computer-aided design (CAD) model. They can therefore produce the optimal porous structures to attain the desired mechanical behavior, permeability and diffusion properties for a given application. Moreover, the CAD model can be obtained from medical scan data (computerized tomography or nuclear magnetic resonance imaging), allowing the external shape of the scaffold to match the damaged tissue site.

Currently, biodegradable scaffolds are processed either from ceramics (calcium phosphates or bioglasses) or from polymers (polylactic and polyglycolic acids, ε-polycaprolactone, polydioxanone, etc.). Ceramic scaffolds show a greater potential for bone tissue engineering applications because of their ability to bond directly to bone tissue and their higher elastic moduli [3,4]. Among the SFF methods capable of building ceramic scaffolds, robocasting (also known as direct write assembly or micro-robotic deposition) is unique in that it uses water-based inks with minimal organic content (<1 wt.%) and requires no sacrificial support material or mold [5–7]. This technique consists of the robotic deposition of highly concentrated colloidal suspensions (inks) capable of fully supporting their own weight during assembly. Thus, a 3-D structure is printed directly as a network of ink rods extruded through the deposition nozzle. With the recent development of robocasting inks made from β-tricalcium phosphate [8] (β-TCP) and hydroxyapatite [9–11] (HA) powders, this technique has allowed customized calcium phosphate scaffolds to be built for bone regeneration.

However, despite the improvement in pore architecture achieved with this SFF method, the main limitation of these ceramic scaffolds still lies in the poor mechanical resistance associated with their porosity [12]. A possible approach to achieving better mechanical performance would be to develop a composite material by infiltration of a biodegradable polymer into the ceramic

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structures. The addition of a polymer phase to a ceramic scaffold has been shown to enhance toughness [13,14] and strength [15,16] in non-SFF scaffolds (i.e. with limited control of pore architecture). Although the toughness enhancement has been attributed to crack bridging by polymeric fibrils [13], which significantly increases the fracture energy, the actual role of the polymer material in the strengthening of the scaffolds remains unclear. Some workers have tentatively suggested that a reduction in scaffold porosity [15] would seem to be the most likely explanation of this effect, but probably because of the intractability of the geometries of their infiltrated scaffolds they did not elaborate any further.

The present work sought to shed light on this question by analyzing the effect of completely filling the macroscopic porosity of a β-TCP robocast scaffold with two different biodegradable polymers, polylactic acid (PLA) and poly(ε-caprolactone) (PCL), by means of infiltration. The controlled geometry of the robocast scaffolds allowed us to explore the effect of polymer infiltration on the stress field within the ceramic structure, using finite element modeling (FEM) simulations of uniaxial compression tests performed on real samples. Fully impregnating the structures fixes the variables porosity and amount of polymer deposited, thereby simplifying the analysis of the results. Furthermore, fully impregnated composite scaffolds might be of interest in themselves, since they could have superior mechanical properties and, given that the bioerosion rate of the polymer infiltrate is greater than that of the calcium phosphate skeleton, one would expect the generation of porosity in situ upon implantation, allowing bone in-growth.

Finally, the use of two different biodegradable polymers with very different mechanical properties provided information about how those properties affect the performance of the composite scaffold. The results provide valuable insight into the mechanical behavior of hybrid robocast scaffolds for bone tissue engineering applications and pave the way for future work aimed at optimizing the mechanical performance of such structures.

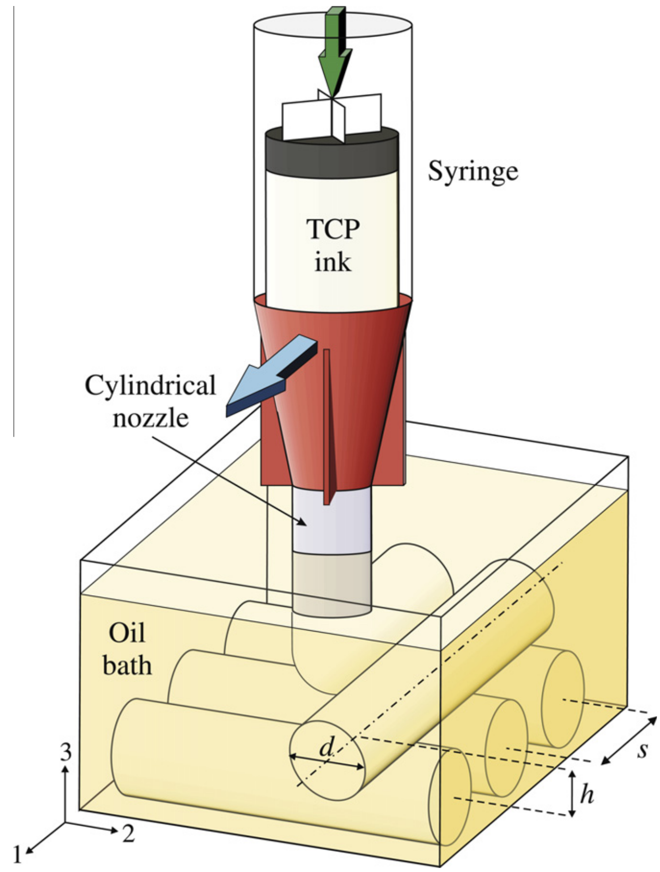

Fig. 1. Schematic illustration of the robocasting fabrication process. The ceramic scaffold is built layer by layer from a computer design. A 3-axis robotic arm moves the injection syringe while pressing the ceramic ink through the cylindrical deposition nozzles, immersed in an oil bath, to create a self-supporting 3-D network of ceramic rods. Relevant dimensions of the scaffolds (rod diameter *d*, rod spacing *s*, and layer height *h*) are indicated.

2. Experimental procedure

2.1. Materials and sample preparation

Commercially available Ca-deficient β-TCP powder (Fluka, Buchs, Switzerland), pre-calcined at 1300 °C to obtain a final 84 wt.% β-TCP/16 wt.% calcium pyrophosphate (CPP) composition [17] and attritor milled to around 1 μm particulate size, was used to prepare inks for robocasting with a final solid content of 40 vol.%, following a procedure similar to that of a previous work [8,12]. First, a stable suspension was prepared by dissolving 1.5 wt.% (relative to powder content) Darvan® C dispersant (R.T. Vanderbilt, Norwalk, CT) in distilled water and gradually adding the β-TCP powder. An appropriate amount (7 mg ml liquid^{-1} in the final suspension) of previously dissolved hydroxypropyl methylcellulose (Methocel F4 M, Dow Chemical Co., Midland, MI) was then added to the mixture to increase the viscosity. Subsequently, the ink was gellified by adding 2 vol.% (relative to liquid content) polyethylenimine (PEI) as flocculant. After each addition the mixture was placed in a planetary centrifugal mixer (ARE-250, Thinky Corp., Tokyo, Japan) for a few minutes to improve its homogeneity and stability.

3-D β-TCP scaffolds consisting of a mesh of ceramic rods were constructed layer by layer via direct write assembly of the ink using a robotic deposition device (3-D Inks, Stillwater, OK) (see Fig. 1). The printing syringe was partially filled with the ink and placed on a 3-axis motion stage, controlled independently by a computer-aided direct write program (Robocad 3.0, 3-D Inks). The ink was deposited through cylindrical metallic deposition nozzles (EFD Inc., East Providence, RI) with a diameter d = 250 μm, at a printing speed of 30 mm s^{-1}. Each layer in the computer 3-D model of the structure consisted of parallel rods with a center-to-center spacing s = 500 μm. Rods in adjacent layers were orthogonal and the spacing between layers was set to h = 200 μm. The external dimensions of the scaffolds were set at about 14 × 14 × 10 mm so that a total of 50 layers were deposited. As shown in Fig. 1, the deposition was carried out in a paraffin oil bath to ensure uniform drying during assembly.

The samples were removed from the bath and dried in air at room temperature for at least 24 h, and then at 400 °C (1 °C min^{-1} heating rate) for 1 h to evaporate the organics. They were finally sintered at 1200 °C (3 °C min^{-1} heating rate) for 1 h to avoid microcracking [17]. At this sintering temperature full densification of the green samples cannot be completely achieved and important residual in-rod porosity (around 25% [17]) was observable in the sintered scaffolds, as shown in Fig. 2. This rod surface micrograph also shows that grain size in the scaffolds was around 3 μm.

Biodegradable polymers were infiltrated into selected scaffolds by immersion of the ceramic structures in a polymer melt. Commercial pellets of PLA (2002D, Natureworks, Minnetonka, MN) or PCL (Capa 6500, Purac, Barcelona, Spain), with average molecular weights according to the suppliers' specifications of around 200 and 50 kDa, respectively, were used. Optimal soaking temperatures and times for infiltration of each polymer were selected by trial and error. Optimal melting temperatures were found to be 227 °C for PLA and 220 °C for PCL. After complete melting of the polymers the TCP scaffolds were immersed and soaked for 2 h and then cooled to room temperature.