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3D printing of porous hydroxyapatite scaffolds intended for use in bone tissue engineering applications

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ABSTRACT

A systematic characterisation of bone tissue scaffolds fabricated via 3D printing from hydroxyapatite (HA) and poly(vinyl)alcohol (PVOH) composite powders is presented. Flowability of HA:PVOH precursor materials was observed to affect mechanical stability, microstructure and porosity of 3D printed scaffolds. Anisotropic behaviour of constructs and part failure at the boundaries of interlayer bonds was highlighted by compressive strength testing. A trade-off between the ability to facilitate removal of PVOH thermal degradation products during sintering and the compressive strength of green parts was revealed. The ultimate compressive strength of 55% porous green scaffolds printed along the Y-axis and dried in a vacuum oven for 6 h was 0.88 ± 0.02 MPa. Critically, the pores of 3D printed constructs could be user designed, ensuring bulk interconnectivity, and the imperfect packing of powder particles created an inherent surface roughness and non-designed porosity within the scaffold. These features are considered promising since they are known to facilitate osteoconduction and osteointegration in-vivo. Characterisation techniques utilised in this study include two funnel flow tests, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), compressive strength testing and computed tomography (CT).

1. Introduction

Bones perform several vital functions within the body; primarily structural support and protection of bodily organs. The ability of this tissue to self-repair and remodel to meet mechanical demands makes it a unique structural composite [1,2]. The capacity of bone to function healthily can, however, be affected by numerous pathological conditions or diseases, and it is also well known that bone degenerates with age [3]. Major alterations in bone structure due to disease or injury can lead to patient discomfort and a reduced quality of life [4]. Defects that fall outside of the capacity of bone to self-repair may require surgical intervention, thus creating a demand for appropriate clinical strategies. However, due to the complex hierarchical structure of bone, this clinical need presents an on-going medical challenge.

Tissue engineering is an interdisciplinary field that aims to combine the knowledge of cells, biomaterials, and suitable biochemical factors to create a surrogate structure to guide and regenerate new tissue [5]. Ideally, the structural component of this strategy, referred to as a scaffold, should be made from appropriate biomaterial(s) and fabricated so as to mimic the physical and chemical structure of the host tissue [6]. Critically cancellous bone exhibits a porous bulk and surface structure that is highly interconnected which enables cell migration, vascularisation and new tissue growth [7–9].

Broadly, scaffold fabrication methods can be grouped as conventional or Additive Layer Manufacturing (ALM) techniques. In recent years ALM methods have received much attention since they enable the user to build in desired levels of hierarchical complexity; which is particularly advantageous when trying to mimic the physical structure of bone. ALM techniques may be divided into three sub-groups: (1) laser or other directed energy beam, (2) print or 'ink', and (3) nozzle systems [10].

The motivation of the presented work is to use a print-based ALM technique, namely 3D printing (3DP), to fabricate customised porous scaffolds suitable for use in bone tissue engineering applications. This ALM method processes powdered materials layer-by-layer using a counter rotating roller to spread a user defined thickness from a platform, moveable along the Y-axis and filled with stock powder (powder bed) to another level area within the printer, again moveable in the Y-axis, where the part is built (build bed). Once a layer of powder has been spread to the build bed a liquid binder is then propelled onto the surface in appropriate areas by a printer head, causing adjacent particles of the same and neighbouring layers to bind together. This process is repeated layer-by-layer until the part is complete. In particular, this ALM method was selected since good cell–biomaterial interaction has been reported for 3D printed parts due to the inherent roughness created because of imperfect packing of powdered stock materials [11,12].

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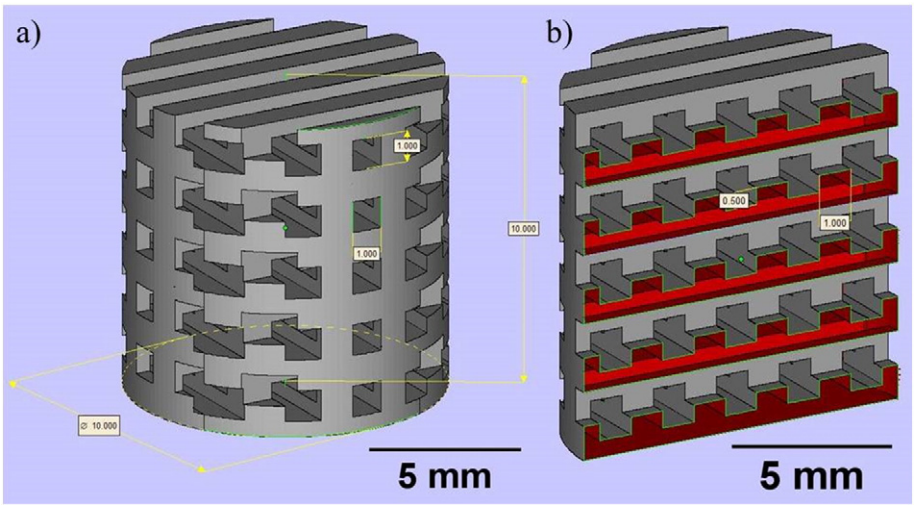

Fig. 1. CAD design of 3DP cylindrical scaffolds (1 mm pores, 10 mm height and diameter). (a) External structure and (b) internal structure.

Due to the chemical likeness of calcium phosphates (CaPs) to bone mineral, in particular hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$– HA], this group of bioceramic materials is commonly used in bone replacement applications [13–18]. A number of authors have reported the fabrication of porous CaP structures by 3DP. Pure CaP powders, for example α- and β-tricalcium phosphates [$Ca_3(PO_4)_2$– TCP] [19], tetracalcium phosphate [$Ca_4(PO_4)_2O$] [20], and HA [21], as well as composites of CaPs mixed with organic polymers, such as poly(L-lactide-co-glycolide)-copolymer (PLGA) [22], have been utilised as stock materials. Combinations that require the use of organic solvents as a binder, for example PLGA and β-TCP bound with chloroform [22], have an intrinsic disadvantage as there is always a risk of finding toxic solvent residues in the 3D printed structure [23]. These studies highlight the versatility of the 3DP method for use with different precursor materials, despite this there is still a need for new biocompatible powder-binder systems that enable accurate printing of porous ceramic scaffolds with sufficient mechanical strength [20]. The CaP phase selected for this work was HA due to its chemical likeness to bone mineral, notably the model could be adapted for use with other CaP powders, such as TCP, which have the advantage for faster dissolution under physiological conditions.

Composite precursor powders of HA and PVOH were utilised in this study to 3D print porous scaffold structures. Prior to printing, a number of techniques were used to assess vital characteristics of the precursors to determine their suitability as stock materials, for example flowability. The physical structure of green and sintered scaffolds was analysed using scanning electron microscopy (SEM), compressive strength testing and computed tomography (CT). Fourier transform infrared spectroscopy (FTIR) was also used to observe any chemical variation of constructs post sintering. This combination of precursor as well as, the green and sintered part characterisation, contributes to the knowledge in this field and highlights key trade-offs between scaffold properties critical to the success of bone tissue implants.

2. Experimental procedure

Purchased PVOH particles (Mowiol 23–88, 150,000 g/mol, Clariant, UK) was ball milled to less than 200 μm prior to dry mixing with HA (>90%, Fluka, UK). A partially hydrolysed grade of PVOH was selected to enable dissolution when wetted with the water-based binder during printing. In particular, grade 23–88 was chosen as a first assessment, notably the selection of a different partial hydrolysed PVOH grade would impact the viscosity of the wetted particles; however, such an investigation is outside the remit of this paper. Prior to scaffold fabrication the printability of various HA to PVOH ratios (HA:PVOH), from 0:100 to 100:0 wt.% was assessed in order to select appropriate materials for the 3DP process.

Scaffolds with 55% porosity were designed in Solidworks CAD software (Dassault Systèmes SolidWorks Corp, USA) and converted to the standard file format for ALM (STL) (Fig. 1). A ZPrinter 310 + 3D printer (ZCorporation Inc., USA; now owned by 3D Systems Inc., USA) was then used to print constructs using a powder layer thickness of 0.1 mm and a maximum binder saturation level.

Green parts were left to dry for 1 h, as recommended by the printer manufacturer, before being removed from the build bed. De-powdering was performed using compressed air directed through a syringe needle attached to an AS18K airbrush compressor (Absolute Airbrush, UK). Green scaffolds were either left as printed, or dried in a furnace or vacuum oven at 60 °C for 2 or 6 h (Table 1). Sample IDs were assigned with the HA content, post-processing method (P — printed, F — furnace dried, V — vacuum dried), the post-processing time in h and printing direction

Table 1
3D printed scaffolds produced from HA:PVOH precursor powders.

HA content (wt.%)	Printing direction	Drying method	Drying time (h)	Sample ID
100–60	N/A			
50	X	N/A — as printed		50PX
		Furnace	2	50F2X
			6	50F6X
		Vacuum	2	50V2X
			6	50V6X
50	Y	N/A — as printed		50PY
		Furnace	2	50F2Y
			6	50F6Y
		Vacuum	2	50V2Y
			6	50V6Y

Table 2
Flowability results for HA:PVOH precursor powders (n = 3). Results quoted as average ± standard deviation / √3.

HA (wt.%)	Flow time (s)	Angle of repose (°)
100	4.7 ± 0.1	38.7 ± 0.2
90	3.0 ± 0.6	35.8 ± 0.5
80	2.9 ± 0.2	35.6 ± 0.4
70	2.3 ± 0.2	35.4 ± 0.4
60	2.0 ± 0.1	27.3 ± 0.4
50	1.6 ± 0.05	25.8 ± 0.4
0 (100% PVOH)	0.5 ± 0.02	21 ± 2.0