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Printability of calcium phosphate: Calcium sulfate powders for the application of tissue engineered bone scaffolds using the 3D printing technique

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ABSTRACT

In this study, calcium phosphate (CaP) powders were blended with a three-dimensional printing (3DP) calcium sulfate ($CaSO_4$)-based powder and the resulting composite powders were printed with a water-based binder using the 3DP technology. Application of a water-based binder ensured the manufacture of CaP:$CaSO_4$ constructs on a reliable and repeatable basis, without long term damage of the printhead.

Printability of CaP:$CaSO_4$ powders was quantitatively assessed by investigating the key 3DP process parameters, i.e. in-process powder bed packing, drop penetration behavior and the quality of printed solid constructs. Effects of particle size, CaP:$CaSO_4$ ratio and CaP powder type on the 3DP process were considered. The drop penetration technique was used to reliably identify powder formulations that could be potentially used for the application of tissue engineered bone scaffolds using the 3DP technique.

Significant improvements ($p < 0.05$) in the 3DP process parameters were found for CaP (30-110 μm):$CaSO_4$ powders compared to CaP (<20 μm):$CaSO_4$ powders. Higher compressive strength was obtained for the powders with the higher CaP:$CaSO_4$ ratio. Hydroxyapatite (HA):$CaSO_4$ powders showed better results than beta-tricalcium phosphate (β-TCP):$CaSO_4$ powders. Solid and porous constructs were manufactured using the 3DP technique from the optimized CaP:$CaSO_4$ powder formulations. High-quality printed constructs were manufactured, which exhibited appropriate green compressive strength and a high level of printing accuracy.

1. Introduction

As the knowledge of material, structural and biological properties of tissue engineered (TE) scaffolds increases, an interconnected porous scaffold to mimic extracellular matrix of living bone has been highlighted as an essential requirement. This has driven the rapid development of solid free-forming (SFF) techniques also termed rapid prototyping (RP), in an effort to produce pre-designed TE scaffolds [1–4].

Powder-based inkjet 3DP is one of the most attractive SFF techniques. It involves a sequential layering process through which 3D porous scaffolds can be produced directly from computer-generated models. Scaffolds manufactured from this process have the advantages of a fully controlled geometry, customized properties and high reproducibility. The 3DP also exhibits great potential for clinical applications due to the capability to make custom-fit scaffolds based on a computer tomography scan of the defect site. In a 3DP process, binder droplets are ejected through ink-jet printhead nozzles and selectively deposited on the powder bed to build a model from a series of cross sections, which correspond to the 3D CAD/CAM design. Many commercial ink-jet 3D printers currently use thermal drop-on-demand printheads. The working principle of thermal printheads involves forming and collapse of liquid bubbles that repeatedly occur on the heating element inside the printhead [5]. The formation of bubbles squeezes droplets out of an array of nozzles. Due to this thermal process, thermal drop-on-demand printheads are limited to use for low-viscosity fluids that do not degrade or corrosively attack the heating elements during vaporizing [6,7]. To date, mainly four types of liquid have been reported to be used as the binder in the 3DP system for processing powdered biomaterials: (1) water solution suspended with polyvinyl materials [8,9]; (2) chloroform solvent [10]; (3) acidic solution [11,12]; and (4) water-based liquid [13–15]. The suspended solids within the in polymeric solution may increase rate of nozzle wear and the chloroform solvent and acid solutions can cause degradation of the heating elements. Use of these liquids in the thermal printhead may reduce the working life of the printhead. Therefore in practice, it is restricted to water-based media (>98% water), which is also the commercially used binder in the 3DP system.

CaP, such as HA and β-TCP, are biocompatible materials exhibiting excellent osteoconductivity due to its chemical similarity to the natural bone. Recently, many attempts have been made to produce CaP

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scaffolds using an ink-jet 3D printer [2,3,12,16–18]. However, in order to print a strong and robust construct, CaP usually requires a setting reaction with an acidic binder, such as citric acid [12], phosphoric acid [18] and tartaric acid [19]. Despite some initial success, use of acidic binder in a thermal ink-jet printhead significantly compromised working life/performance of the printhead [6]. This drawback seriously limits the large-scale manufacturing of CaP materials via the thermal ink-jet 3DP system and may pose a challenge for the uptake of this novel technique in tissue engineering.

In this study, a new method was proposed in an effort to increase the printability of CaP powders using the thermal 3DP technique. The method involved blending the CaP powder with an additive powder, which must be biocompatible, biodegradable and be able to facilitate formation of a solid phase of suitable handling strength via a reaction with water. Therefore it enables a reaction with the water-based medium and eliminates the requirement for an acidic binder. In this study, $CaSO_4$ was chosen, for combining with the CaP phase, due to its excellent printability in thermal ink-jet 3DP manufacturing [4]. Mixing $CaSO_4$ with water activates a self-hydration reaction that leads to recrystallization into a solid form of gypsum ($CaSO_4 \cdot 2H_2O$) [20]. Since its first clinical report in the repair of bony defects in 1892, $CaSO_4$ has been proved as an effective substitute for bone graft [21–23]. It has been proven to enhance new bone formation, undergoes complete *in vivo* resorption and does not increase the extent of the inflammation reaction post-implantation [24]. However since 1990s, the use of $CaSO_4$ in orthopedic applications has been gradually substituted by HA due to two main drawbacks: (1) low strength and (2) rapid resorption [25]. Nevertheless, new research interest has been shown towards the use of HA:$CaSO_4$ as bone substitute material and good results have been obtained [26,27].

Prior to the 3DP manufacturing of constructs from a new powder formulation, it is important to review characteristics that influence the 3D printability of this powder. The term '3D printability' was first defined by Butscher et al. [3] as 'powder characteristics essential for the 3DP process'. It is mainly influenced by two powder characteristics: (1) powder topology, including particle size, particle size distribution, morphology and specific surface area and (2) material reactivity with the binder, which in this study was referred to as the reactivity with water-based binder. Among the powder topological characteristics, particle size has been demonstrated as the dominant factor in determining the quality of printed products [28]. Different studies have found different particle size range for efficient 3DP construct manufacture. Lu et al. [28] has suggested that higher strength for 3DP constructs prior to any hardening post-process (i.e. green strength) can be achieved using fine powder (<20 μm), whereas other studies found fine powder caused issues with spreading during the 3DP process [3,29]. These contradictory results indicate lack of understanding on this emerging manufacturing technology, especially on the fundamental principles of how the binder droplets interact with the powder during the 3DP process.

Simply stated, the powder-droplet interaction involves three steps: (1) droplet penetration into powder pores, (2) wetting adjacent powders and (3) setting to form nucleation [30]. McEleney et al. [30] identified some of the crucial factors influencing powder-droplet interaction: binder spread **behavior**, powder-binder wettability and reactivity. It is postulated that these factors play an important role in determining the successful application of a powder using the 3DP technique. Therefore, it is wholly justified to evaluate the powder-binder interaction prior to the process. In this study, the effects of particle size, CaP:$CaSO_4$ ratio and type of CaP powders on the 3D printability were investigated using the drop penetration technique with the aim of identifying new protocols to assist in the screening of new powder formulations for the 3DP technique. Consequently solid and porous constructs were manufactured using the 3DP technique based on the **optimized** CaP:$CaSO_4$ powder blends defined using the drop penetration technique.

2. Material and methods

HA (Capital®, Plasma Biotal Ltd., UK) and β-TCP (Whitelockite, Plasma Biotal Ltd., UK) were used as the CaP powders in this study. The true particle density for the HA and β-TCP powders was 3.156 g cm^{-3} for HA and 3.140 g cm^{-3} (Table 1), which was provided by the material supplier (Plasma Biotal Ltd., UK).

The HA and β-TCP powders were milled and sieved according to the standard techniques used by the powder supplier (Plasma Biotal Ltd., UK). Subsequently, the supplier ball milled the powders and then used particle elutriation to ensure the particle size and distribution corresponded to the authors' requirements. The particle size distribution (D_{10}, D_{50} and D_{90}) of the coarse HA and β-TCP powders was carefully selected to be comparable to the 3DP $CaSO_4$-based powder (ZP102, Z Corporation, UK) used in this study [4]. Particle size distributions were measured using a two laser Sympatec HELOS/BF Particle Sizer (Sympatec Limited, UK). The powder was scanned in triplicate to obtain the average of D_{10}, D_{50}, and D_{90} values, which are representative of the mean particle size for 10%, 50%, and 90% of the material.

HA and β-TCP powders of different particle size were investigated and then categorized into two groups: fine particle and coarse particle (Table 1). For the fine particle groupings, HA and β-TCP powders exhibited a $D_{90} < 20$ μm. The coarse particle groupings demonstrated a $D_{10} \geq 20$ μm. Each of the four CaP powders was blended with the $CaSO_4$ powder at CaP:$CaSO_4$ ratios of 25:75 wt.% and 50:50 wt.%. Blending of each composite powder was conducted three times using a High Speed Mixer (Rondol, UK) at rotational speed of 1600 rpm for a 2 min period. Previous studies by the authors have demonstrated that the adoption of this technique and protocol is appropriate to ensure a homogenous dispersion of the powders [31,32]. In total eight different composite powder combinations were produced (Tables 2 and 3).

The powder packing for each CaP:$CaSO_4$ composite powder was determined by measuring the in-process powder bed density and bulk density. Two custom-made reservoirs (150 mm × 150 mm, with adjustable height) were placed in the feed and build area in a thermal ink-jet 3D printer (Zcorp 310, Z Corporation, UK). The reservoirs were designed for the purpose of reducing the amount of powder required for the 3DP process and also to assist in the measurement of the powder bed density. Specifically, the reservoir in the feed area was filled with a composite powder prior to the 3DP process. During the process, a relative small component (0.1 mm × 0.1 mm × 10 mm) was printed, which added negligible mass to the powder bed. Consequently the powder was spread from the feed area to the other reservoir located in the build area. Following this step, the reservoir in the build area was removed from the 3D printer. The mass of powder within the reservoir and height of the powder bed were measured and subsequently used to determine the in-process powder bed density. The powder bulk density was quantified in accordance with the ISO Standard 10545–3 [33]. Additionally the powder bed packing ratio was calculated (Eq. (1)).

$$\text{Powder bed packing ratio} = \text{Powder bed} \times \frac{\text{density}}{CaP \times \rho_{CaP} + \text{CaSO}_4 \times \rho_{CaSO_4}} \tag{1}$$

Table 1
Powder properties for starting CaP and $CaSO_4$ materials.

Powder Properties	$CaSO_4$	HA		β-TCP	
		Coarse	Fine	Coarse	Fine
D_{10} (μm)	28.12	45.68	1.02	32.63	6.22
D_{50} (μm)	67.84	65.35	2.33	54.50	13.04
D_{90} (μm)	101.83	94.40	3.49	109.29	19.92
True Particle Density ρ (g cm^{-3})	2.760	3.156	3.156	3.140	3.140