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Moisture based three-dimensional printing of calcium phosphate structures for scaffold engineering

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ABSTRACT

Powder based three-dimensional printing (3DP) allows great versatility in material and geometry. These characteristics make 3DP an interesting method for the production of tissue engineering scaffolds. However, 3DP has major limitations, such as limited resolution and accuracy, hence preventing the widespread application of this metho engineering. In order to reduce these limitations deeper understanding of the complex interactions between powder, binder and roller during 3DP is needed. In the past a lot of effort has been invested to optimize the powder properties for 3DP for a certain layer thickness. Using a powder optimized for an 88 μm layer thickness, this study systematically quantifies the surface roughness and geometrical accuracy in printed specimens and assesses their variation upon changes of different critical parameters such as the moisture application time (0, 5, 10 and 20 s), layer thickness (44 and 88 μm) and the number of specimens printed per batch (6 and 12). A best surface roughness value of 25 μm was measured with a moisture application time (using a custom made moisture application device mounted on a linear stage carrying the print head) of 5 s and a layer thickness of 44 μm. Geometrical accuracy was generally higher for the 88 μm thick layer, due to a less critical powder bed stability. Moisture application enabled 3DP of a 44 μm thick layer and improved the accuracy even for a powder initially optimized for 88 μm. Moreover, recycling of the humidified powder was not only possible but, in terms of reactivity, even beneficial. In conclusion, moisture-based 3DP is a promising approach for high resolution 3DP of scaffolds.

1. Introduction

Three-dimensional printing (3DP) is a versatile solid, free-form fabrication (SFF) technique with high potential for scaffold engineering. The flexibility that 3DP offers is outstanding not only in terms of geometrical flexibility (free-form) but also in terms of the broad materials choice [1]. Provided the desired material exists in powder form of appropriate size, almost any material can be synthesized by 3DP. This flexibility opens up totally new approaches within regenerative medicine, such as 3-D printed patient-specific models based on calcium phosphate (CaP) powders for maxillofacial bone regeneration [2–4]. While CaP is well established as a synthetic bone substitute biomaterial [5–8], scaffolds built up by 3DP of CaP powders provide a unique geometrical flexibility that cannot be achieved by traditional manufacturing processes. Furthermore, the inherent rough powder surface in 3DP is reported to enhance cell adhesion [9–12].

However, as high as the potential of 3DP is, the challenges are and will be large. The geometrical flexibility is restricted by the limited resolution (the typical layer thickness is close to 100 μm). While for certain industrial 3DP applications this resolution might be sufficient, it is critical when building up tiny and complex geometries for scaffold engineering. The definition of an adequate pore size is still a matter of debate [13]. However, it is generally reported to be in the range 50–1000 μm [14–16]. While high resolution free-form fabrication methods (e.g. stereolithography) allow the production of pores in the lower size range, only macropores larger than 500 μm can be currently achieved using 3DP [1].

The resolution and accuracy of 3DP are determined by multiple factors, such as print head resolution, precision of the linear stage positioning, binder drop volume, binder–powder interaction, particle size and, last but not least, layer thickness. Unfortunately, efforts to determine these effects are often hampered by the limitations set by commercial printers. Nevertheless, even if a printed specimen meets the required accuracy, depowdering (removing loose particles around and within the printed body) is critical.

In order to achieve a breakthrough in 3DP for scaffold engineering, accuracy (the mismatch between the model and the 3DP specimen) and resolution (smallest feature size) need to be

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substantially improved [17]. In a previous work the interplay between powder properties and final printing outcome was investigated in detail [18]. It was demonstrated that particle properties such as particle size, flowability, wettability and compaction rate could be optimized and thus determine the best possible 3DP outcome for a certain layer thickness. In particular, it was shown that there was an optimum range of powder flowability related to the powder mean particle size. When the powder was too fine flowability was too low, thus resulting in smearing and poor resolution. When the powder was too coarse flowability was too high, leading to powder bed instability and again poor resolution.

Currently the resolution of 3DP is mainly limited by too large a layer thickness, typically in the region of 100 μm. This provokes the so-called stair stepping effect [19], which has a significant impact on the surface texture of printed solids. While stair stepping is predominant for curved surfaces, a similar effect is detected on vertical surfaces due to small lateral shifts occurring between superimposed layers. A smaller layer thickness is thus mandatory. However, the ideal 3DP powder should have a mean diameter greater than 20 μm because below this value interparticular forces dominate gravitational forces [20]. Furthermore, the layer thickness should be at least three particles thick, due to issues of powder flow and spreadability [21]. In other words, there is a conflict between higher accuracy on the one hand and 3-D printer and powder requirements on the other.

In the literature a few studies have shown that powders smaller than 20 μm can be controlled to a certain degree by plasma coating [22] or lubricants [23]. However, these changes, in connection with a change in particle size [24], have an impact on the wetting characteristics of the powder. Other studies have shown that layer thicknesses down to 25 μm are possible [25,26], without, however, focussing on the link between layer thickness and printing accuracy. Powder bed stability during deposition of a new layer also becomes a very important issue at low layer thicknesses and small particle sizes. Indeed, since shear forces in the powder bed increase with a decrease in layer thickness, recoating of a new powder layer is not only a complex but critical factor [27,28]. Furthermore, the binder droplets jetted onto the powder bed are more likely to displace small particles than large particles. In order to improve the powder bed stability local application of moisture to the powder bed was chosen. This method has already been mentioned in a few articles by the inventors of 3DP [29,30], however, an in depth understanding of this approach is lacking.

This study aims at systematically analysing the interplay between layer thickness, layer stability and the final quality of the printed specimens. In order to approach the resolution and pore sizes relevant for scaffold engineering layer thicknesses of 88 and 44 μm were selected and compared. Moreover, tests were carried out without and with local moisture application to the top powder layer, in order to enable and improve 3DP with the fine powders necessary for 44 um layers.

Based on the insights gained it is hoped that 3DP resolution and accuracy can be improved, which in return would lead to the synthesis of more accurate CaP scaffolds.

2. Materials and methods

2.1. Powders

A home-made α-tricalcium phosphate (α-TCP) (α-$Ca_3(PO_4)_2$) powder was used. α-TCP is a more reactive phase than β-TCP and leads to better 3DP results [18]. Furthermore α-TCP has excellent in vivo behavior [31]. In our approach α-TCP particles undergo a cement reaction described in detail in Butscher et al. [1]. Briefly, the α-TCP particles are dissolved by the phosphoric acid print head jet and brushite crystals precipitate in the resulting solution. Hardening (or particle "binding") occurs via entanglement of these brushite crystals. Accordingly, and for convenience, the phosphoric acid solution is termed "binder" in this manuscript. Further densification of the reacted powder can be achieved using either heat treatment or improved by a post-hardening regime after printing, also improving the in vivo biological stability [32].

The powder was produced according to a previously published procedure [26] differing only in the use of a lower Ca/P molar ratio (1.45) and slightly higher sintering temperature (1400 °C). Briefly, a 0.45:1 M ratio blend of calcium carbonate (CC) powder ($CaCO_3$, Merck, Germany, catalogue No. 102076) and dicalcium phosphate (DCP) powder ($CaHPO_4$, GFS Chemical, USA, catalogue No. 1548) was mixed in a Turbula mixer (Bachofen, Switzerland) for 1 h. After calcining at 900 °C for 1 h in an LHT 02/16 furnace (Nabertherm, Germany) the powder was cooled to room temperature. The calcined powder was then sieved (AS 200, Retsch, Switzerland) through a 0.125 mm sieve, sintered at 1400 °C for 4 h, and then removed from the furnace to quench the powder in air. Finally, the sintered powder was broken in a jaw crusher (BB51, Retsch, Germany), milled and separated to obtain the desired particle fraction (according to Butscher et al. [18]).

2.2. 3D printer

A commercial 3-D printer (Zprinter 310plus, Z Corp., Burlington, USA) was used. The printer was adapted to meet the requirements of this study. Firstly, the feed and build reservoirs were reduced to areas of 60 × 90 and 50 × 80 mm, respectively, to enable 3DP with smaller powder amounts and thus reduce the time per print and the powder material cost. A layer thickness of 88 μm and a binder/volume ratio of 0.28 for the shell and 0.14 for the core of the 3DP using pure 10% phosphoric acid as the binder fluid (or reaction fluid) were taken to be standard parameters. Unfortunately, printing a layer thickness less than 88 μm was not possible with the commercial version of the printer used in this study. Therefore, a relatively simple mechanical approach was used to halve the layer thickness. To this end the linear actuators used to control the vertical position of the feed and build volume were disassembled and the original lead screws with an outer diameter of 9.525 mm and a thread lead of 2.54 mm were replaced by an equivalent lead screw and matching threaded sleeve with half the thread lead. To print a layer thickness of 44 μm the binder/volume ratio was kept the same as mentioned above. The exact layer thickness was determined using a digital displacement gauge (S229 dial gauge, Sylvac, Switzerland) for at least six steps and repeated three times.

Furthermore, a custom made moisture unit was mounted on the linear stage (Fig. 1a). This moisture unit allowed application of moisture via a magnetic inlet valve just before the print head jet applied the binder to the powder bed. Pure water moisture was produced inside a nebulizer (USV 3002, Schulte, Germany) normally used for inhalation therapy. The moisture was applied from the top of the moisture chamber and was sucked away via channels inside the walls of the moisture unit by a ventilation system (Fig. 1b). A custom made control system allowed automated positioning and timing of the moisture unit within the 3DP process.

In order to quantify the effect of different moisture regimes and layer thicknesses simple solids of spheres, cubes, cylinders (Fig. 2a) and pyramids (described later) were generated with the CAD software NX 7.5 (PLM Software, Siemens, Germany) and imported into the 3-D printing software in the .stl (stereolithography) file format. Since the printer was unaware of the hardware changes the models had to be scaled according to the change in linear travel of the actuator. While the sphere represented a critical case concerning powder bed stability, the cube and cylinder, which are less critical,

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