

SOLID FREEFORM FABRICATION OF CALCIUM POLYPHOSPHATE DUAL-POROUS STRUCTURE OSTEOCHONDRAL SCAFFOLD

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Abstract

In this work the solid freeform fabrication (SFF) of dual-porous structure scaffolds using the 3D-printing method is investigated. The structure, including a cartilage substrate and a bone scaffold with different porosities and pore sizes, provide a suitable facility for repairing the osteochondral tissues in the implanted site. Calcium polyphosphate (CPP), with distinct particle sizes for each portion, was utilized as the biomaterial. Polyvinyl alcohol (PVA), as a biocompatible polymer was also used as a binder to adhere the CPP particles upon injecting of a solvent through the 3D-printing process. The prototyped parts are finally post-processed in the controlled furnace to obtain the required bio-mechanical properties. The biomechanical properties of the fabricated samples are also characterized by the X-ray diffraction (XRD), scanning electron microscopy (SEM), and density analysis.

Introduction

Tissue engineering consists of interdisciplinary fields that apply the principles and methods of engineering and life sciences toward the development of biological replacements that restore, maintain, or improve tissues function [1, 2]. In other words, tissue engineering aims at producing patient-specific biological substitutes to overcome the problems in the existing clinical treatments for damaged tissues such as the lack of donor organs [3]. Scaffolds play a critical role in tissue engineering. The primary regenerative tissue engineering approach involves the transplantation of cells onto scaffolds. They mimic the function of the natural extracellular matrices for cell accommodation, proliferation, and differentiation. Also, scaffolds serve as three dimensional templates for new-tissue formation [4].

Recently, as a tissue engineering approach, efforts have been focused on developing new treatments in biological reparation that includes the implantation of a degradable temporary osteochondral scaffold followed by the regeneration of the damaged tissue [5]. For example, the bioengineering of an articular cartilage biphasic construct which comprises a bone-interfacing component is under progress by researchers [6]. For this purpose, as a newly developing method, scaffolding with a single but heterogeneous structure is attracting the attentions [7].

Calcium polyphosphate (CPP) has exhibited the potential to be a reliable candidate for building biodegradable osteochondral scaffolds. CPP has been proposed as a bone implant

material as porous rods. The studies have demonstrated that sintered CPP is well accepted by the surrounding tissue upon implantation [8].

Recently, solid freeform fabrication (SFF) has been received interests for fabrication of complex-shaped implants and scaffolds. So far, the preliminary experiments conducted by using the 3Dprinting method, as one of the SFF techniques, ascertain the feasibility of processing biomaterials, including the bio-polymer and bio-ceramic powders for fabrication of scaffolds [9].

In this work, the fabrication of a heterogeneous structure, called dual-porous scaffold, is considered. The dual-porous structure, consisting of a top thin substrate with smaller pores and a scaffold with larger pores, is utilized for in vitro culturing of the cartilage on the top substrate and in vivo bone ingrowth within the scaffold. CPP, which is available in powder form, is selected for the material. The scaffold fabrication is conducted by using the 3D-printing method.

Materials and Method

CPP glass powders with a particle size of fine (45-75 μ m) and coarse (106-150 μ m) were utilized in this study. For the 3D-printing of CPP, supplementary materials are required as binder and solvent. The polyvinyl alcohol (PVA) powder, 98-99% hydrolyzed, medium weight (Alfa Aesar, Ward Hill, MA, USA) were ground and sieved (U.S. standard sieve series, Sieve No. 140) in a range smaller than 63 μ m, to be used as the binder material. The composite powder was utilized in two different types: (1) mixed and (2) coated. For the mixed one, 10w% PVA was mixed with CPP for 4h by using a roll jar mill (US Stoneware, Ohio, US), whereas the CPP particles were coated with a thin layer of PVA through the super-saturation drowning-out technique [10] for the latter one. An aqueous solvent was used in this study.

A commercial 3D-printing machine, ZPrinter310-Plus (Z Corporation, Burlington, USA), was employed in this study to fabricate the scaffolds. The experiments were conducted on the fabrication of CPP cylindrical scaffolds with single and dual-porous structures. The specimens were designed as circular discs with diameters and height of 4mm. The cylindrical samples are prototyped from two types of starting powder: fine (45-75 μ m) and coarse (106-150 μ m). For each type of powder, a specific layer thickness was adjusted such that it was greater than the particle size.

In addition, dual-porous scaffolds were fabricated by using the PVA-mixed CPP (45-75 μ m) for the top substrate and PVA-coated CPP (45-75 μ m) for the bottom scaffold. The powder feeding chamber of the 3D-printer was filled with fine powder followed by the coarse powder. Thus, the sample was built in the sequential layers starting from coarse scaffold and ending with dense substrate.

The 3D-printed parts must have enough green strength to be transferred to the post-processing stage in which the parts are sintered. The polymeric binder retains the net shape of the sample. In the post-processing stage, the polymer decomposes and leaves the structure. The necks generate between the particles through sintering process. This is conducted in a platinum crucible using an air furnace (Thermolyne® 48000, Barnstead International, Dubuque, Iowa

USA). The heat treatment conditions for the binder removal and gravity sintering processes are summarized in Table 1.

Table 1: Post-processing parameters for different samples

Sample	Debinding Step	Sintering Step
Fine CPP	500°C	950°C
Coarse CPP	500°C	950°C
Coated CPP	400°C	950°C
Dual-Porous	500°C	950°C

Analysis and testing

The following experimental tests were conducted on the produced samples.

1. X-ray Diffraction

The X-ray diffraction (XRD) technique (Philips PW) was used to identify the various phases of the CPP sintered parts. The XRD tests were conducted by using a step scan in which the angle (2θ) was set between 20 and 90 with a step size of 0.02.

2. Micro-Structure Examination

Micro-structure of the sintered samples were assessed by using scanning electron microscopy (SEM) (JSM-6460, Jeol Ltd., Akishima, Tokyo) to determine the surface morphology, pore size, shape, and the inter-particle sinter necks.

3. Porosity Measurement

The density of each sintered sample was determined by the Archimedes principle according to ASTM C373 with a density measuring kit (Sartorius YDK01, The Sartorius Group, Goettingen, Germany).

To determine the density/macro-porosity percentage of the samples, the bulk density of the sintered samples is compared with the theoretical density of the non-porous CPP (2.85 g/cm^3) [11], as follows:

$$\text{Density} = \frac{\rho_{\text{bulk}}}{2.85} \times 100\%$$

$$\text{Porosity} = \left(1 - \frac{\rho_{\text{bulk}}}{2.85}\right) \times 100\%$$

4. Shrinkage Measurement

The dimensional shrinkages in both diameter and height directions are determined by measuring the samples before and after sintering in the furnace. Considering the dimensional changes in the 3D-printing stage and shrinkage during the sintering, an anisotropic correction factor can be obtained to compensate for the dimensional mismatches between the CAD design and the final sample.

Results and Discussion

The fabricated cylinder after sintering is shown in Figure 1. The cylinder has retained its geometry unchanged during the sintering. Figure 2 illustrates the 3D-printed dual-porous structure CPP sample.



Figure 1: Solid freeform fabricated CPP cylinders after sintering

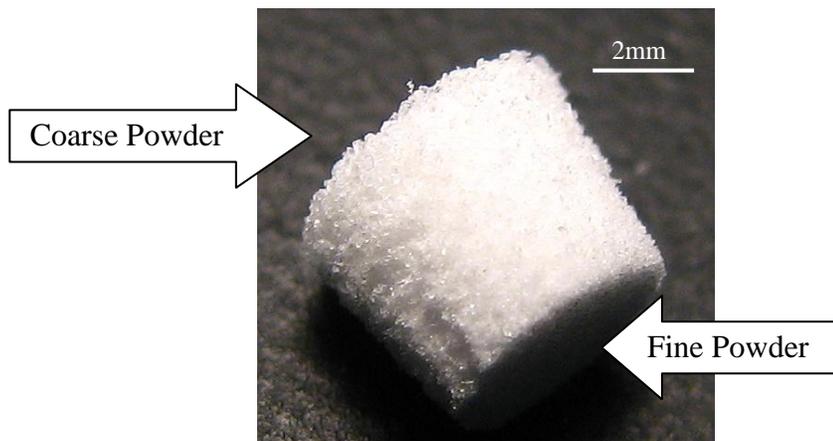


Figure 2: Dual-porous structure CPP sample before sintering

Figure 3 demonstrates the XRD patterns of the crystalline CPP samples, sintered at 950°C. The similarity in the variation of the relative intensity on the XRD patterns for the sintered samples and samples of [12] indicates that the sintered CPP contains a β -CPP phase. In addition, a comparison of the XRD for the CPP samples, fabricated with and without the binder, confirms

that there is no negative effect from the PVA and the solvent. The prepared starting CPP powders are typically amorphous.

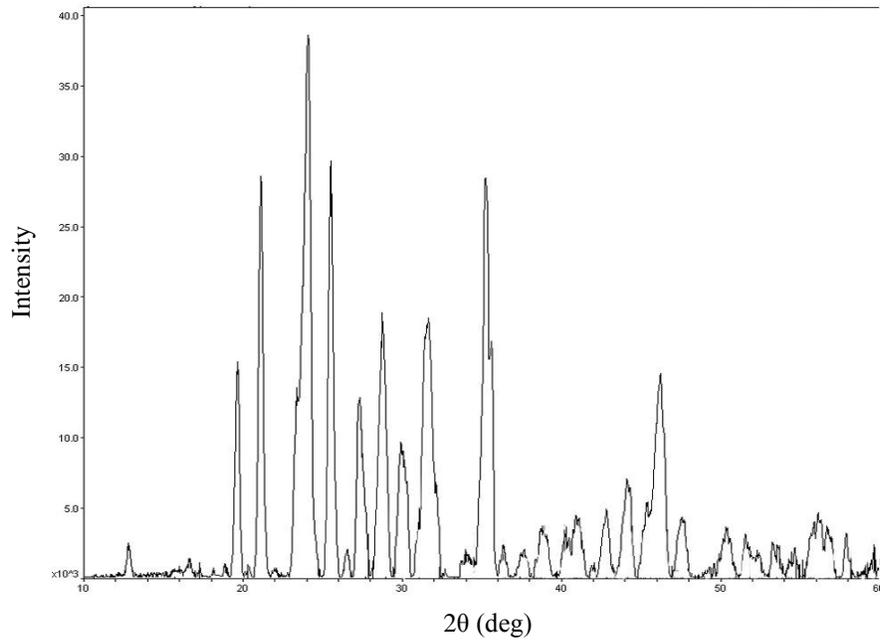
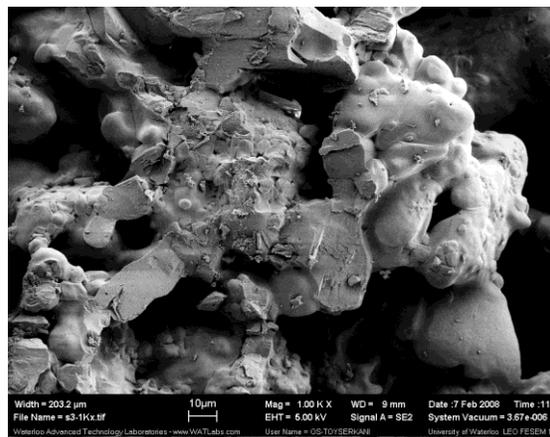
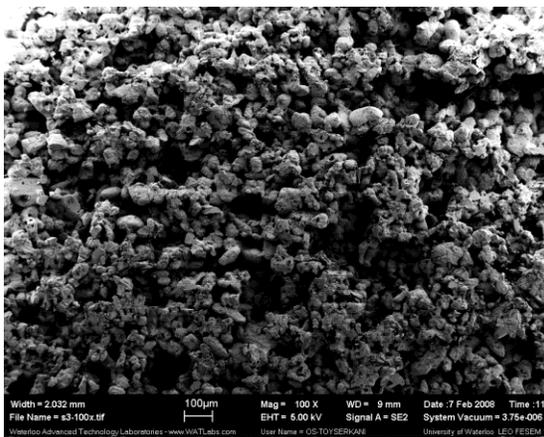
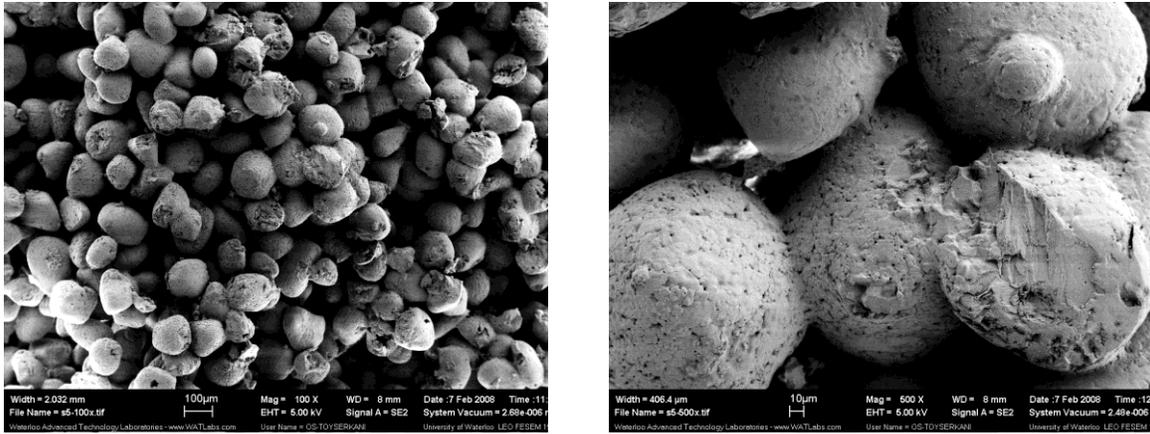


Figure 3: XRD of the SFF prototyped CPP

Figure 4 (a)-(b) are SEM pictures of the cross-sections of the sintered structures for the fine and coarse CPP powder at the low and high magnifications. The micro-pores of about $10\mu\text{m}$ are obvious on the particle surface which is the result of the sintering. Macro-pores between $50\text{-}200\mu\text{m}$ are observed within the samples.



(a)



(b)

Figure 4: SEM of the sintered parts with different particle sizes: (a) fine powder (100X) and (1000X), and (b) coarse powder (100X) and (500X)

The SEM of the cylinders fabricated with the PVA-coated CPP is demonstrated in Figure 5. The properly developed necks are obvious in the SEM picture that confirms the success of using coated CPP.

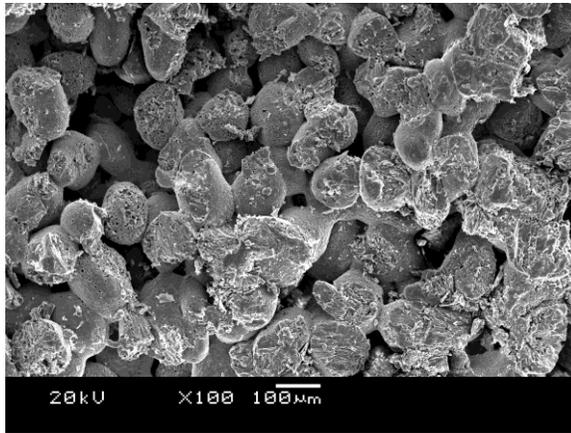


Figure 5: SEM of the fabricated part with PVA-coated CPP (100X)

The Micro-structure of dual-porous structure CPP constructs is reflected in Figure 6. The 1mm top substrate with the fine particles is distinctive. A thin transition region is composed of both fine and coarse powders. The top substrate includes pores in the range of 30-50 μm , whereas the scaffold portion has 50-200 μm pores.

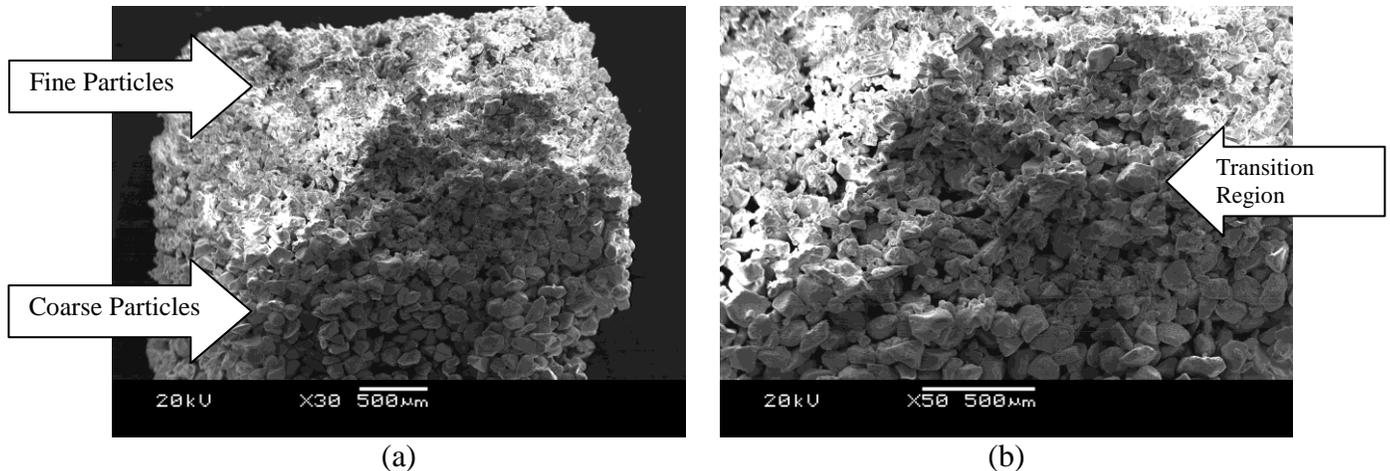


Figure 6: SEM of the sintered dual-porous CPP sample: (a) 100X and (b) 50X

The parts which were prototyped by the PVA-coated CPP powder indicated a large dimensional deviation from the CAD model. The 3D-printed cylinders are 34% greater in diameter, whereas this difference averages about 15% and 11% for the fine and coarse mixed CPP-PVA powder, respectively. It can be concluded that the surface tension between the solvent and the coated powder is much higher than that of the mixed powder which causes more infiltration of the solvent.

On the other hand, the constructs shrink during sintering. For the same sintering conditions, the fine and coarse powders demonstrate a 14% and 7% sintering shrinkage in diameter, respectively. It is measured 12% for the coated CPP. The calculations indicate that the parts shrink with almost the same values in height.

The average volume densities are measured approximately 50% for the fine and coarse CPP samples, respectively. However, it is observed that the density increased for the coarse samples fabricated with the PVA-coated CPP to 60%. It should be mentioned that the different debinding and sintering temperature definitely have contributed to the increase of the density as well. The values mean a porosity of approximately 50-40%, which is suitable for osteochondral scaffolds.

The results, in addition, suggest that open pores predominate within the structure, which is also confirmed by the SEM images. The interconnectivity of pores is necessary for cell nutrition.

Conclusion

The SFF of single and dual-porous structure CPP scaffolds was conducted by using different CPP powder particle sizes. The supplementary materials which were used in the 3D-printing of the scaffolds do not cause any contamination or phase change in the CPP samples. Investigating the micro-structure and the density of the prototyped scaffolds demonstrates that the SFF prototyped scaffolds involve the appropriate macro-pore size, distribution, and interconnectivity. It shows that the scaffolds may be suitable for osteochondral tissue engineering applications. The fabricated dual-porous structures consist of a top substrate, with smaller pores

proper for cartilage *in vitro* culturing on that, and a scaffold, with larger pores for bone *in vivo* ingrowth.

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