

## PHYSICAL PROPERTIES CHARACTERIZATION OF POROUS CALCIUM PHOSPHATE SCAFFOLD FOR BONE TISSUE ENGINEERING

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### Abstract

The porous calcium phosphate scaffold is used to repair bone defect. Cracked during pyrolysis process is the main problem of the porous calcium phosphate due to the low mechanical strength and high brittleness. This lead to reduce efficiency of the scaffold for cells proliferation. The aim of this work is to improve the mechanical properties of these ceramic scaffolds. Green body was produced using foam replication technique. The polyurethane foam with dimension of (20 x 10 x20) mm was immersed in slurry of apatite powder and poly vinyl alcohol (PVA). To obtain porous scaffolds, the green body was treated under various sintering temperature i.e. 1150°C, 1250°C and 1300°C for 3 hours, followed by dipping in PDLLA solution and dried at room temperature for 24 hours. The specimen was analysed under X-ray diffraction, SEM and compressive strength. The X-ray diffraction spectrum shows the presence of  $\text{Ca}_{10}(\text{PO}_4)_6\text{OH}_2$  phases in the sintered scaffold. The formation of rod-like apatite was observed through SEM. The compressive strength was increased with increasing sintered temperature. It has a potential to be used as tissue engineering for low-load bearing application.

Keywords: Porous calcium phosphate scaffold, replication technique, green body

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## 1.0 INTRODUCTION

Calcium phosphate ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) is the major component for bone replacement. It can be used to regenerates or repairs tissue, in the event of organ or tissue loses or failure occurs [1]. To serve as porous scaffolds, the material must have excellent biocompatibility, osteoconductive and no swelling or shape distortion after implantation. However their mechanical properties such as compressive strength is very poor especially when the porosity is high. To avoid the brittleness of porous ceramics, ceramic composite systems has been developed. Ceramic scaffolds are important to control the porous structural parameters such as porosity, pore size, and pore interconnectivity for bone tissue engineering. Several techniques have been investigated to obtain the required porous scaffolds [3]. The foam technique is the one which offers a great flexibility in controlling the pore size with several desired complex shapes and straightforward. The foam replica technique involved coating of open-cell polymeric

foam with ceramic slurry followed by burning out polymeric foam through sintering process which a replica of the original polymer foam in the ceramic foam structure.

For bone tissue engineering, the design of scaffold should mimic the structure and properties of the bone extra-cellular matrices, because bone consists of porous composite of interpenetrating phases of hydroxyapatite and collagen. The scaffolds for bone regeneration should be similarly porous composite with interpenetrating ceramic and polymer phases. Calcium phosphate scaffolds often facing a problem of the pore wall, which often cracked during pyrolysis process due to low mechanical strength and high brittleness [2]. To solve this problem, the ceramic scaffold must be coated with a polymer layers, intended to fill existing cracks in the structure of the bioceramic.

In this study, the ceramic calcium phosphate based scaffolds were coated with PDLLA to improved mechanical properties of porous structure. The pore size and morphology of scaffold were

characterized using scanning electron microscope (SEM). X-ray diffraction (XRD) was used to characterize the crystal structure and chemical composition.

## 2.0 EXPERIMENTAL

The apatite powder used for starting materials were synthesized using calcium hydroxide (FLUKA) and phosphoric acid (Mallinckrodt) and spray dried to obtain the apatite powder. Polyvinyl alcohol (PVA), 99% hydrolysed (Mw= 85,000-124,000) used as a binder was purchased from Sigma-Aldrich. Double-distilled water was used throughout the study.

The porous scaffolds were prepared using polyurethane (PU) foams infiltrated apatite slurry. Dissolving 2.5g of PVA in 50ml DDI at 80°C. Apatite powder were added and stirred for at least 1 hour. The PU foam with dimension of (20 x 10 x 20) mm was immersed in apatite slurry for 2 minutes and the extra slurry was squeezed out vigorously by hand. The green body was then oven dried at 60°C for overnight. The burning out of the PU foam was done at 550°C for 2 hours with heating rates at 2°C/min. The sample was sintered at 1150°C, 1250°C and 1300°C for 3 hours with cooling rate of 5°C/min.

To improve the mechanical properties of the sintered porous ceramics, PDLLA was introduced into the remaining open pores to form a coating on the pore walls of the samples. For the infiltration of PDLLA, the PDLLA was dissolved in DMC (dimethyl carbonate) at temperature of 60°C to form 5 w/v% of PDLLA solution. The porous ceramics were immersed into the PDLLA solution, followed by drying at room temperature for 24 hours to increase the strength of coating.

### 2.1 Characterisation

#### 2.1.1 Porosity

The porosity of the scaffolds was calculated from the bulk density of the scaffold ( $\rho_{bulk}$ ) and the theoretical density of calcium phosphate ( $\rho_{CaP} = 3.14 \text{ g/cm}^3$ ), by the equation (1) below:

$$\text{Porosity} = \left[ 1 - \frac{\rho_{bulk}}{\rho_{CaP}} \right] \times 100\% \quad (1)$$

The dimension and the weight of each sample were measured and recorded using a Vernier calliper and analytical balance, respectively. Five specimens were used to determine the total porosity.

#### 2.1.2 Microstructure Analysis (SEM, XRD)

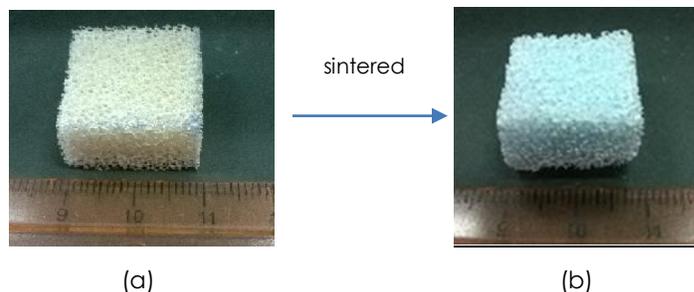
The crystallographic structure of the sintered sample and formation of apatite was performed using X-ray diffraction (XRD) Bruker D8 Advanced. The data was collected over the range of  $2\theta$ . The microstructure of the sample was performed using SEM.

#### 2.1.3 Mechanical Testing

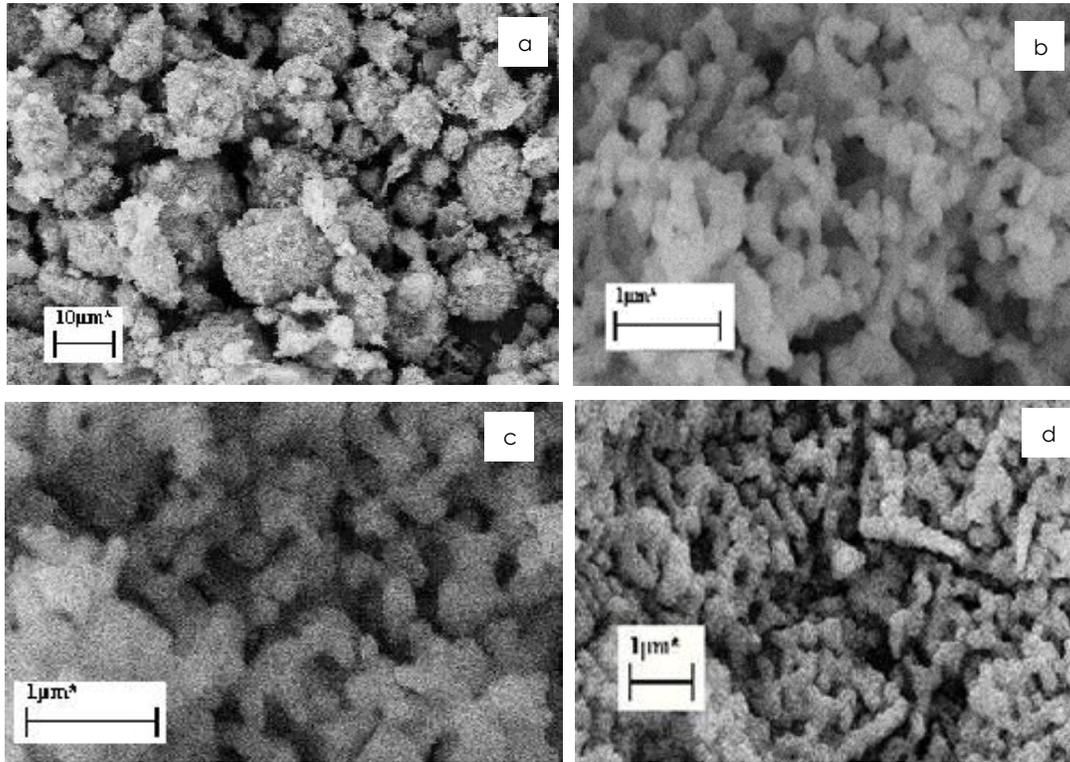
The compressive strength was determined using Universal Tensile Machine (UTM) with crosshead speed of 2 mm/min with the sample of (20 x 10 x 20) mm. At least five samples were tested to get average results.

## 3.0 RESULTS AND DISCUSSION

The scaffolds for bone regeneration should have a homogenous interconnected porous structure with good mechanical properties to withstand loading during bone formation [6]. A sintered ceramic scaffold using foam replication technique was shown in Figure 1.



**Figure 1** Porous ceramic body before (a) and after sintered (b), via foam replication method using apatite slurry

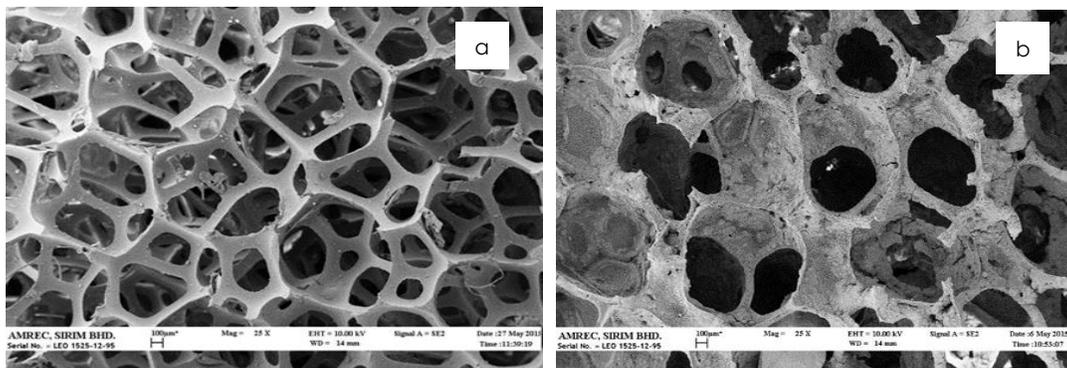


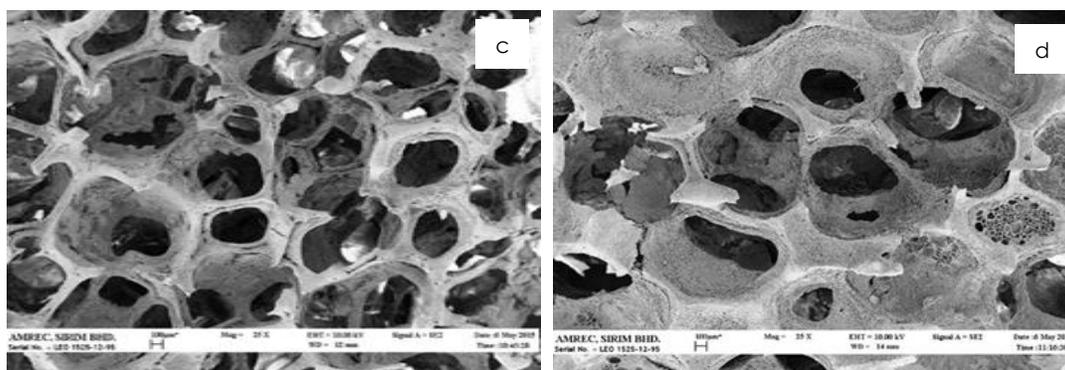
**Figure 2** SEM micrographs of surface powder before (a) and after sintered at (b) 1150°, (c) 1250°C and (d) 1300°C

The typical microstructure of calcium phosphate powder, before sintered (Figure 2a) and after sintered at different sintering temperature, were shown at Figure 2b, 2c and 2d. It can be seen that the pore wall morphology changed obviously, with the formation of bone like apatite formed after the sintering process.

Figure 3a below shows the original sponge network of the polyurethane foam with pore 45 ppi. The effects of sintering temperature (1150 - 1300°C) on sintered microstructure of the micrographs is shown in

Figure 3b-3d. The similar reticulated structure was obtained; the presence of a few microcracks was observed in the reticulated struts, which occurred during pyrolysis of the sponge template. With increasing sintering temperature from 1150 to 1300°C, average pore of calcium phosphate scaffolds were decreased in the range of 415 – 330 μm, an increase of pore density has contributed the number of struts. Therefore, the optimal pore size needed for bone tissue engineering proposed to be within 100-800 μm [4], for new tissue formation.





**Figure 3** Microstructure of (a) PU foam, and struts microstructure of sintered at (b) 1150°C, (c) 1250°C and (d) 1300°C

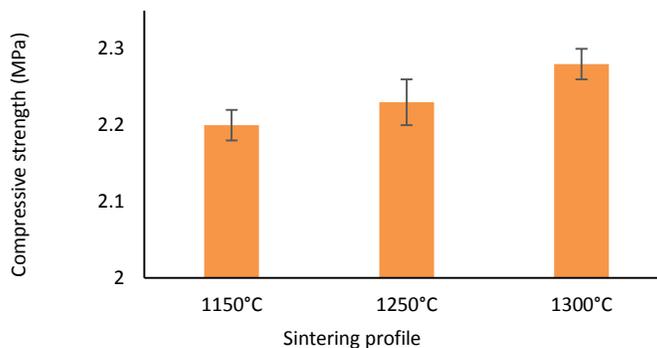
The porosity and pore size of scaffolds play a critical role in bone formation *in vitro* and most provide good condition for cell growth and flow transit of nutrients and metabolic waste, because of their higher specific surface area. In this case, the total porosity values of scaffolds was around 93% - 91%, respectively. The open pore structure of sintered scaffold was retained after coating with PDLLA solution, with only a few of the pores being blocked by the coating. Porous ceramic scaffold with high mechanical properties generally have low porosity will provide the opportunities for cell and tissue growth.

Compressive strength data related to sintering temperature as shown in Table 1 and Figure 4. After sintering of porous calcium phosphate scaffolds at 1150°C, the strength obtained is around  $2.2 \pm 0.02$

MPa and increases to  $2.23 \pm 0.03$  MPa at 1250°C and  $2.28 \pm 0.02$  MPa at 1300°C. The compressive strength increases with the sintering temperature which corresponds to the increasing of samples density and decreasing of porosity. The obvious increasing in the strength was due to reduce porosity at this range of sintering temperatures. For tissue engineering applications, the high mechanical strength of porous scaffolds together with high porosity is desirable; however, it is difficult technically since the mechanical strength of materials generally behave in an opposite manner to porosity [5]. The obvious increase in the strength was due to the large reducing of porosity at this range of sintering temperature.

**Table 1** Physical properties at various sintering temperatures

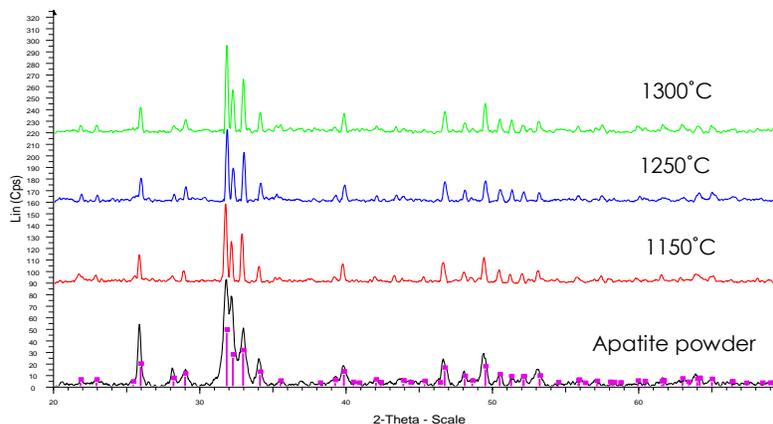
Sintering Temp (°C)	Compressive Strength (MPa)	Density (g/cm <sup>3</sup> )	Porosity (%)	Pore size (µm)
1150	$2.2 \pm 0.02$	$0.16 \pm 0.05$	93	415
1250	$2.23 \pm 0.03$	$0.20 \pm 0.01$	92	384
1300	$2.28 \pm 0.02$	$0.84 \pm 0.04$	91	330



**Figure 4** Compressive strength of calcium phosphate scaffold with different sintering profile

The crystalline phases of the scaffolds were investigated by XRD analyses, as shown in Figure 5. The results revealed that the reproducibility of crystalline hydroxyapatite (HA) peaks was achieved. All these batches shows similar pattern and there were no impurities detected. These results confirmed

that thermal processing at up to 1300°C did not alter the crystalline structure of the material. The behaviour of calcium phosphate apatite after increasing crystallinity due to specific high temperature.



**Figure 5** XRD spectra of Calcium phosphate powder before and after sintering at increasing temperature

## 4.0 CONCLUSION

The calcium phosphate porous scaffolds were fabricated using foam replication method and polyvinyl alcohol (PVA) as binder and PDLLA as a coated polymer base. Scaffolds possessing the necessary requirement for use in bone tissue engineering (93% -91% porosity, 415-330  $\mu\text{m}$  pore size) were produced from a fully crystalline powder. Porosity and compressive strength of calcium phosphate scaffold were much affected by the sintering temperature. Higher sintering temperature gave better strength to calcium phosphate scaffold that is associated with the decreasing in porosity and also the formation of the struts.

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## References

- [1] S. A. Goldstein and M. R. Moalli. 2001. Current Concepts in Tissue Engineering: Cell, Matrices, and Genes. *Cur. Opin. Orthop.* 12: 424-427.
- [2] Darmawati Mohamad Yunos, Oana Bretcane. 2008. Polymer-bioceramic Composite for Tissue Engineering Scaffolds. *J. Mater. Sci.* 43: 4433-4442.
- [3] I. Sopyan, M. Mel, S. Ramesh, K. A. Khalid. 2007. Porous Hydroxyapatite for Artificial Bone Applications. *Science and Technology of Advanced Materials.* 8: 116-123.

- [4] Qianbin Wang, Qiguang Wang, Changxiu Wan. 2011. The Effect of Porosity on the Structure and Properties of Calcium Polyphosphate Bioceramics. *Ceramic-Silikaty*. 55(1): 43-48.
- [5] Joo-Hyeek Lee, Hong-Jun Choi, Seog-Young Yoon, Byung-Kyu Kim and Hong-Chae Park. 2013. Processing of Porous Hydroxyapatite Scaffolds Containing Calcium Phosphate Glass-Ceramic for Bone Tissue Engineering. *Journal of ceramic Processing Research*. 14(4): 544-548.
- [6] Hassna Rehman Ramay, Miqin Zhang. 2003. Preparation of Porous Hydroxyapatite Scaffolds by Combination of the Gel-Casting and Polymer Sponge Methods. *Biomaterials*. 24: 3293-3302.
- [7] Julian, R. Jones, Larry, L. Hench. 2003. Regeneration of Trabecular Bone Using Porous Ceramic. *Solid State & Materials Science*. 301-307.
- [8] Joo-Hyeek Lee, Hong-Jun Choi, Seog-Young Yoon, Byung-Kyu Kim and Hong-Chae Park. 2013. Processing of Porous Hydroxyapatite Scaffolds Containing Calcium Phosphate Glass-Ceramic for Bone Tissue Engineering. *Journal of ceramic Processing Research*. 14(4): 544-548.
- [9] M. Dressler, F. Dombrowski, U. Simon, Bornstein, V. D. Hodoroba, M. Feigi, S. Grunow, R. Goldenhaar, M. Neumann. 2011. Influence of Gelatin Coating on Compressive Strength of Porous Hydroxyapatite Ceramic. *Journal of Europe Ceramic Society*. 31: 523-529.
- [10] Jon-Hoon LEE, Hyoun-Ee KIM and Young-Hag KOH. 2010. Fabrication of Porous Calcium Phosphate Cements Using Gelatin as Porogen. *Journal of ceramic Society of Japan*. 118(1): 34-36.