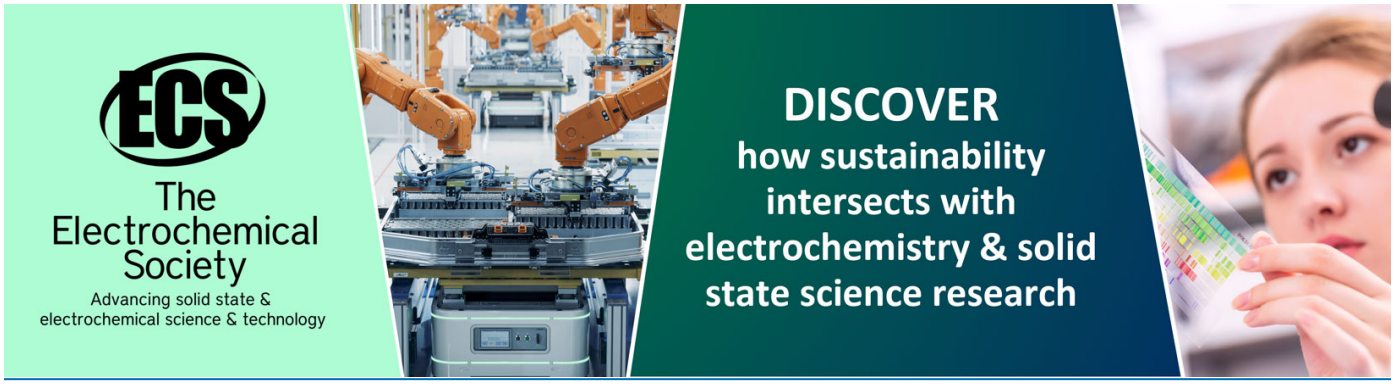
Effect of Freezing Temperature on The Pore Formation of Beta Tricalcium Phosphate Scaffold



Effect of Freezing Temperature on The Pore Formation of Beta Tricalcium Phosphate Scaffold

M. Muhammad Iqbal[1], I. Nurul Fatin Nabila[1], and A. Nurazreena[1]*

[1]Niche Biomaterials, School of Materials and Mineral Resources Engineering, Engineering, Campus, Universiti Sains Malaysia, 14300 Nibong Tebal, Pulau Pinang, Malaysia

Email: nurazreena@usm.my

Abstract. Beta-tricalcium phosphate (β-TCP) has been widely used for biomedical application due to its excellent mechanical strength, good tissue compatibility and outstanding chemical stability. There are several methods to fabricate porous scaffolds including solvent casting, phase separation, rapid prototyping and electrospinning methods. In the present study, β-TCP scaffolds were fabricated using freeze drying method. β-TCP slurry was frozen and freeze dried for 24 hours followed by sintering process to form β-TCP scaffolds. The porous β-TCP scaffold were fabricated at different percentages of β-TCP powder used (10 wt. %, 20 wt. % and 30 wt. %) and froze at different freezing temperature (-10°C, -20°C, -40°C and -80°C) in order to study the effect of these parameters on the pore formation of the scaffolds. Scanning electron microscopy (SEM) analysis for 30 wt. % β-TCP scaffold frozen at -10°C revealed acceptable pore size distribution with majority pore size within the range of 102.65 µm to 372.90 µm. Furthermore, this scaffold also possessed highest compressive strength of 0.31 MPa.

1. Introduction

Bone tissue is a living organ which mainly composed of both organic and inorganic component. Generally, bone consists about 10% to 20% of water and approximately about 60% to 70% bone mineral. The remaining essential material existed in living bone is collagen with small trace amounts of proteins and inorganic salts. Typically, living bone is widely recognized organ that capable of forming highly functionalized connective tissue [1]. To ensure the bone receives enough supply of minerals that able to prevent breakage of bone, this connective tissue also serve as mineral reservoir for essential minerals such as calcium and phosphorus. All these functions demand for a balance and healthy bone system [2]. Over the years, there are various type of different clinical alternatives used for treating bone defects including autologous and allogeneic transplantations using autografts and allografts. Despite numerous benefits, the disadvantages of allografts and autografts comprised of possibility of disease transmission such as human immunodeficiency virus (HIV) and Hepatitis, limited supply of donor tissue, complication to the donor site and formation of scar after surgery [3]. Decades ago, a noval approach to bone reconstruction has been widely discovered and developed. Which today known as bone tissue engineering. Among the tissue engineering technique, a three-dimensional (3D) scaffold offer temporary structural support during bone reconstruction for cell infiltration and act as a physical support to lead the cell activity into the targeted tissues or organs seems to be the most promising technique for the

treatment of bone defects [4]. In this research, beta Tricalcium phosphate (β-TCP) porous scaffold were prepared via freeze drying technique to investigate the effect of β-TCP powder loading and freezing temperature on the pore formation. β-TCP was chosen as the bone substitute material because β-TCP are known for its ability to bond with living tissues and it has been reported that β-TCP shows excellent biological performance with surrounding host tissues.

2. Experimental study

2.1 Preparation of β-TCP scaffold

The porous bioceramic β-TCP (Sigma Aldrich, Germany) scaffolds were fabricated by freezing of tricalcium phosphate (TCP) slurries. The slurries were first prepared by mixing 5 wt% of gelatine (Halagel, Malaysia) with distilled water. The mixture was stirred using magnetic stirrer to ensure homogenous mixing until the temperature reached 60°C. The slurries were then mixed together with β-TCP powders and 0.25 wt. % of polyvinyl alcohol (PVA) (Merck, Germany) which act as a binder. After the addition of PVA and β-TCP powder, the suspension was homogenously mixed by using mechanical stirrer for 15 minutes at a speed of 300 rpm. Three different slurries were prepared respectively with 10 wt. %, 20 wt. % and 30 wt. % β-TCP powder for each different freezing temperature. Afterwards, the homogeneous slurries were de-aired using a vacuum desiccator for 10 minutes with pressure supplied of 0.01 MPa to remove trapped air bubbles. The mixture was then stirred by using mechanical stirrer at a speed of 200 rpm and the gelatine cross-linking agent, 0.25 wt. % of glutaraldehyde solution (Merck, Germany) was added into the solution and continuously stirred for one minutes. In order to study the effect of freezing temperature on the pore formation of the scaffold, the slurries were casted into cylindrical polyvinyl chloride (PVC) mold and froze in a freezer at different temperature of -10°C, -20°C, -40°C and -80°C for 24 hours. The frozen samples were then freeze dried using LYOQUEST-55 freeze dryer at very low vacuum pressure which was 0.05 mBar and at low temperature of -50°C for 24 hours.

2.2 Sintering of the β-TCP scaffold

After freeze drying process was completed, the porous of β-TCP scaffold was sintered in an air furnace. The sintering process involved two stages, the first stage involved low initial heating rate which was 5°C/min to reach temperature of 500°C. Heating at 500°C was held for 2 hours to ensure complete burn out of polymer substances. The second sintering stage was performed at 1100ºC for 6 hours. After 6 hours, the temperature cooled down to room temperature at rate of 3°C/min.

2.3 Characterization and analytical techniques

The starting material and the produced scaffolds were characterized by X-ray diffraction (XRD) analysis. The XRD pattern were recorded with a diffractometer system (X-ray Diffractometer D8 Advance BRUKER-binary V3) using 1.5406Å at 40 kV on all phase of sintered β-TCP powders. Meanwhile, the diffraction angles (2°) were set starting from 10° to 90°.

Morphology of the scaffold were observed using Scanning Electron Microscope (SEM) (Hitachi TM3000, Japan). As these specimens are non-conductive, a conductive layer coating was coated on each specimen prior to observation.

Universal Testing Machine (Series 5982, Instron, USA) was used to determine the mechanical properties of HAp scaffold incorporated with gelatine using 5 kN load cell with a cross head speed of 1 mm per min.

Porosity of the sintered β-TCP scaffolds were measured by using Archimedes' principle according to the Equation 1.

$$Porosity\ (\%) = \frac{(Mw-Md)}{(Mw-Ms)}\ x\ 100\% \qquad Equation\ 1$$

3. Results and discussion

3.1 X-Ray Diffraction (XRD) Analysis

The standard pattern of XRD was obtained from the Joint Committee on Powder Diffraction Standard (JCPDS) database and β-TCP in accordance with ASTM file number of 09-0169. Figure 1 compares the results obtained from the primary XRD analysis of non-sintered β-TCP powder, β-TCP powders sintered at 1100°C and reference β-TCP powder. The high and narrow peaks shown in the spectrum of each XRD patterns imply the presence of high crystallinity β-TCP phases. From the comparison of the patterns, it can be seen that all the diffraction peaks positions match well with the reference XRD pattern of β-TCP. Based on this analysis it is confirmed that single phase β–TCP was formed without presence of any secondary phase. Therefore, these results suggest that the crystallographic structure of β-TCP was not affected by the sintering process.

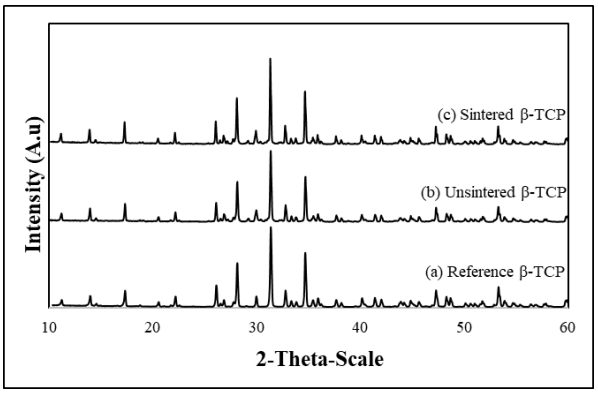


Figure 1. XRD patterns of (a)standard reference of commercial β-TCP; (b) raw unsintered β-TCP; (c) sintered β-TCP 1100°C

3.2 Morphological analysis of β-TCP scaffold

The results of the SEM analysis can be categorized into two parts: 20 wt. % and 30 wt. % of β-TCP powder. β-TCP scaffold with 10 wt. % of β-TCP powder were unable to proceed with the following characterization due to its brittleness. This is because the composition of β-TCP used was minimal and thus resulted in significant reduction in mechanical properties as shown in Figure 2. From the SEM images in Figure 3, the pore size of each scaffolds was calculated and the pore size obtained are shown in Table 1. From Table 1, 20 wt. % of β-TCP powder scaffolds has bigger average pore size than 30 wt. % of β-TCP powder. The pore size of both scaffolds is within the preferably pore size ranges for cell and nutrients transportation in bone tissue engineering (BTE) applications. It can be observed that a significant difference between these two groups of scaffolds whereby for β-TCP scaffold with 30 wt. % of β-TCP powder were having smaller pore when compared to β-TCP scaffold with 20 wt. % of β-TCP powder as shown in Figure 2.

Another important finding is that, from Table 1 it is clearly shows a decreasing trend in pore size from higher freezing temperature (-10°C) to lower freezing temperature (-80°C) regardless the amount of β-TCP powder used. This result may be explained by the fact that lower freezing temperature cause rapid nucleation rate of ice crystal and poor rate of growth throughout freezing stage. These factors contribute to speedy crystallization of solvent into smaller crystal. Subsequently, the small porous sizes were left behind during sublimation of ice crystal during freeze drying [5].

Another possible reason for variation in pore size formation is due to the difference in the fabrication temperature is the difference proximity of void formation during freezing stage and final pore size. As can be seen from Table 1, smaller pores size is obtained at lower freezing temperature (-80°C) and larger

pore size is obtained at higher freezing temperature (-10°C). It can also be observed that large void space forms at -80°C and less void space are formed at -10°C as shown in Figure 4.

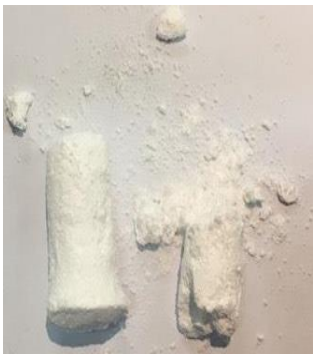


Figure 2. β-TCP scaffold with 10 wt. % of β-TCP powder

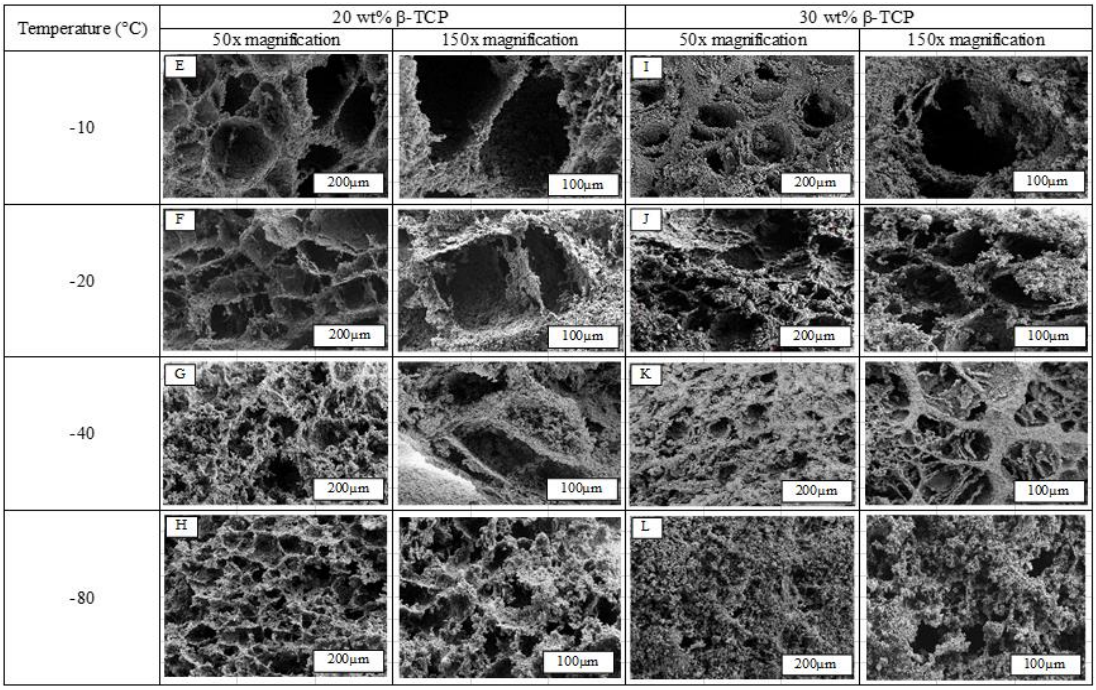


Figure 3. SEM images of β-TCP scaffolds at difference freezing temperature and percentage of β-TCP contents

Table 1. Average pore size of 20 wt. % β-TCP and 30 wt. % β-TCP

Composition of β-TCP (wt. %)	Freezing Temperature (°C)	Pore size (µm)
20	-10	570.76
	-20	343.52
	-40	267.98
	-80	171.97
30	-10	372.90
	-20	210.45
	-40	136.97
	-80	102.65

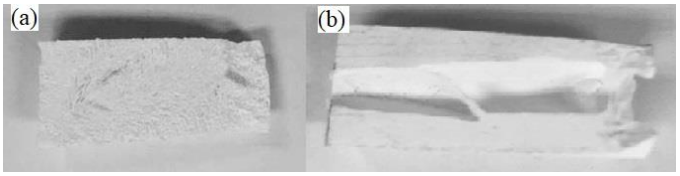


Figure 4. Void formation in scaffold at difference freezing temperature (a) -10°C and (b) -80°C

3.3 Compressive strength of β-TCP scaffolds

From Figure 5 it can be observed that at both β-TCP powder content, similar decreasing trend in the strength of the scaffolds as the freezing temperature decreases. Although it can be noticed that β-TCP scaffolds with 30 wt. % of β-TCP powder has higher compressive strength varying from 0.14 MPa to 0.31 MPa when compared to β-TCP scaffolds with 20 wt. % of β-TCP powder content. The range of compressive strength for β-TCP scaffolds with 20 wt. % of β-TCP powder are from 0.08 MPa to 0.13 MPa. These differences in mechanical strength for both scaffolds may be due to variation in percentage of porosity. The porosity measurement for scaffolds with 20 wt. % of β-TCP powder were 83% while for β-TCP scaffolds with 30 wt. % of β-TCP powder has only 72% porosity as reported in Table 2. It is known that porosity of scaffolds is inversely proportional to the mechanical properties. Therefore, as the number of pores increases, the strength of the scaffold decreases. This finding are consistent with other researchers which found that increased in porosity cause severe reduction in mechanical properties [6].

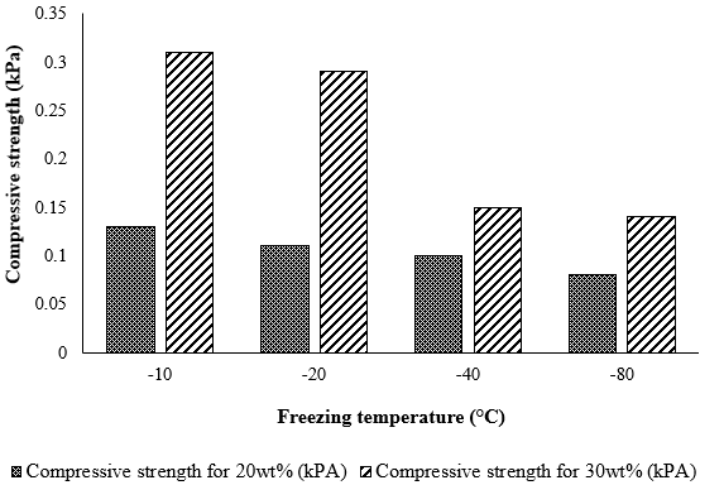


Figure 5. Compressive strength of porous β-TCP scaffolds at different freezing temperature

The observed decreases in compressive strength could be attributed to void formation. This study shows that at lower temperature of -80°C, large central voids are identified. These large voids formation act as stress concentration points that have higher tendency to initiate failure of the scaffolds upon subjected to additional loading [7]. It is therefore likely that such void formations exist within microstructure of scaffold can alter the mechanical properties.

Table 2. Average porosity of β-TCP scaffolds

Composition of β-TCP (wt. %)	Freezing Temperature (°C)	Average porosity (%)
20	-10	82.77
	-20	82.50
	-40	83.45
	-80	83.47
30	-10	71.56
	-20	72.45
	-40	71.86
	-80	73.14

4. Conclusions

Characterization and analysis of 10 wt. % of β-TCP scaffold cannot be conducted since it was too fragile and brittle for handling. In term of pore size of the scaffold and their corresponding effect on both porosity and mechanical properties, SEM analysis performed revealed that smaller average pore size from 30 wt. % of β-TCP powder content is better for bone regeneration compared to 20 wt. % of β-TCP powder content. The SEM results also indicate that the pore size gradually increases as the freezing temperature increased. Compressive strength analysis showed that mechanical properties for 30 wt. % of β-TCP powder content were better than for the 20 wt. % of β-TCP powder content. The compressive strength value increases significantly as freezing temperature decreased. Therefore, scaffold with 30 wt. % of β-TCP powder content fabricated at -10°C freezing temperature yields highest mechanical properties of 0.31 kPa.

5. Acknowledgement

This work was supported by Fundamental Research Grant Scheme (FRGS) No: 203/PBahan/6071302.

References

[1] G. A. Rodan 1992 *Bone* 13 S3
[2] R. Florencio-Silva, G. R. da S. Sasso, E. Sasso-Cerri, M. J. Simoes, and P. S. Cerri 2015 *Biomed Res. Int.* 2015 421746
[3] P. Vang 2006 *Significance* 82
[4] K. Ikeda and S. Takeshita 2014 *J. Bone Metab.* 21 163
[5] L. Jiang, Y. Li, C. Xiong, S. Su, and H. Ding 2017 *ACS Appl. Mater. Interfaces* 9 4890
[6] G. Hannink and J. J. C. Arts 2011 *Injury* 42 S22
[7] A. Zhang, D. Li, H. Lu, and D. Zhang 2011 *Mater. Des.* 32 4803