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# MICROSTRUCTURE OF HYDROXYAPATITE SCAFFOLDS: EFFECT OF INFILTRATION METHOD AND POLYURETHANE TEMPLATE PORE SIZE

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*Porous ceramic scaffolds are widely studied in the tissue engineering field due to their potential in medical applications as bone substitutes or as bone-filling materials. In this study, porous hydroxyapatite (HA) was produced via polymer replication method. Polyurethane (PU) sponge was selected as the template and synthetic binder, polyvinyl alcohol (PVA) was used in this study. Fixed formulation of HA powder, distilled water and PVA (40:60:3) were prepared and stirred at a constant 4 hours time. PU sponges with 30 ppi and 60 ppi size were cut and impregnated in slurry using vacuum and roller infiltration methods. The microstructures were observed by using field emission scanning electron microscope (FESEM). The results obtained indicate that vacuum infiltration method and 60 ppi template pore size exhibited the highest compressive strength with moderate average strut thickness and lowest average pore size compared to samples produced by roller infiltration method at different template pore size.*

**Keywords:** porous hydroxyapatite, polymer replication method, polyurethane

## INTRODUCTION

Scaffold plays an important role in the bone replacement cell attach and colonize. Scaffold serves as a template for cell interactions and formation of new tissue [1,2]. Therefore, the scaffold should behave like true bone in order to optimize integration into surrounding tissue. The design of the bioceramic scaffolds must be extremely precise and compatible with significant chemical and physical resemblance to the mineral constituents of human bones and teeth. Scaffold produced using polymer replication method strongly depends on the structure of polymeric template. The scaffolds may achieve a significantly richer in porosity. Nevertheless, such an abundant porosity is associated to a very high value of brittleness, which undermines the manageability of the scaffold surface. According to Young Yang et al. [3], the production of porous ceramics using polymeric sponge is a simple and low cost process, but the porous ceramic produced by this method are often extremely fragile making them difficult to handle even after sintering and densification.

Hydroxyapatite (HA) is used as main body to produce porous ceramic scaffold fabrication. Scaffolds produced by HA suitable with human body and serve as template for cell interactions and formation of new tissue. The pores in porous HA is the main factor that contribute to the properties of HA scaffolds. The porosity in the scaffolds must be controlled in order to get a good behaviour of scaffolds. Therefore, by varying the size of open porosity in polymer template (PU foam), the pore size, pore size distribution and microstructure of sintered porous HA scaffolds will be changed. Other than that, method to ensure all the slurry infiltrate into the template is very important.

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This is to help the ceramic slurry flow into the polymeric sponge and increase subsequently the strength of ceramic green body [4,5].

The aim of this paper is to investigate the effect of infiltration method by using roller and vacuum infiltration on the microstructure of HA scaffolds. The size of the template is varied to identify the effect of this factor to the properties of HA scaffolds.

## Materials and Methodology

### Materials

Raw materials used in this study were hydroxyapatite (HA) powder, polyvinyl alcohol (PVA) and polyurethane (PU) sponge. HA was used as main body in porous HA scaffold fabrication. HA was purchased from Sigma Aldrich Corporation in form of powder with the mean particle size of 75µm. PVA is the main binder used for the preparation of this ceramic slurry. PVA was obtained from Sigma Aldrich in the form of powder. PU sponge was used as a template of porous HA structure. The sponge was purchased from CCT Automation Sdn. Bhd. PU sponge with a complex pattern decahedron repeated in three dimensions was used as pore former of porous ceramic fabrication. PU foam with 30 pores per inches (ppi) and 60 pores per inch (ppi) was used in this work.

### Methodology

Porous HA scaffolds was fabricated by using replication of polymeric sponge technique. The slurry was prepared with fixed composition of 40 wt% of solid HA loading and 60 wt% of water contents. PVA is used as a binder in this experiment based on 3 wt% from amount of HA loading. The 40:60 ratio between HA solid loading and water create a high viscosity slurry due to high shear resistance that hold the HA particles onto the wall of template. PVA was mixed with distilled water (60 wt %) and stirred with the mechanical stirring at 1600 rpm. This process must be controlled in order to homogenize the mixture. HA powder was added to the mixture and stirring process is continued for 4 hours.

PU sponge used in this study were first cut into a small rectangular shape with dimensions of 25 mm (length) x 25 mm (width) x 25 mm (thickness). Once the polymeric sponge and the ceramic slurry have been prepared, the next step is to impregnate the sponge with the slurry. There are two infiltration methods used in this study to impregnate the slurry into the sponge which are roller infiltration method and vacuum infiltration method. For both method, the sponge was compressed to remove air and then soaked in the slurry for about two minutes to make sure that the slurry completely fills the structure of sponge. Then, they were squeezed to remove the excess slurry leaving pores with interconnected structure.

For roller infiltration method, the sponge was passed through a simple roller to impregnate the slurry into the pores. The distance between two rollers is fixed at 2 mm distance. The soaking, squeezing and rolling step were repeated three times to make sure the slurry fully filled the sponge pores. Whereas for vacuum infiltration method, the sponge was put in vacuum desiccator for 30 minutes after soaking and squeezing process. The vacuum environment is used to make sure that the air trapped inside the sponge's pore was removed and fully filled with HA slurry. The sponge need to be squeezed again to avoid the agglomeration of slurry inside the sponge. The low gas pressure inside the vacuum will create free flow of ceramic slurry inside the polymeric sponge. The soaking, squeezing and vacuum processes for vacuum infiltration method were also repeated for three times. Then, the impregnated PU sponge was dried in air for 24 hours before proceed to another process. After impregnation process, the infiltrated sponges were dried. Drying was done in oven at 80˚C for 24 hours to remove all the moisture inside the sponge.

The sintering process was carried out at the temperature of 500˚C for 60 minutes. After sintered, the sample shrank and became smaller from the original dimension. In this stage, controlled heating was required to prevent the collapse of sponge ceramic frame. The heat treatment was scheduled with heating rate 5˚C/min. The samples were soaked in the furnace at 1250˚C for 60 minutes to obtain porous ceramic structure.

Compression test was carried out in order to investigate mechanical properties of porous HA specimens. Compressive strength of specimens was measured by using INSTRON 3366 with series IX control system. ASTM (C773-88) was used as a standard for this compression test. Field Emission Scanning Electron Microscope (FESEM) (Model: Hitachi TM3000 Table Top) was used to analyze the morphology of the porous HA scaffolds.

## Results and Discussion

### Effect of Infiltration Method and Template Pore Size on the Properties of HA Scaffolds.

Two different infiltration methods (vacuum and roller impregnation methods) were used to investigate the properties of the HA scaffolds. The critical region for the slurry to go inside the sponge is at the centre of sponge because of high energy and fluidity is required to travel across the struts and pores. In this work, two different sizes of PU sponge based on pore per inch unit were used. The sponge pore size is categorized as the important parameter for the selection of template. This is because the porosity of final ceramic foam will be determined based on the template pore size.

Fig. 1 shows the result obtained from compression test analysis of HA scaffolds prepared by two impregnation methods with pore size of 30 ppi and 60 ppi. From Fig. 1, it is found that samples prepared by vacuum infiltration method exhibit higher compressive strength than those of roller infiltration samples. Both samples of 30 and 60 ppi shows the same trend. When comparing the samples prepared by 30 ppi sponge, vacuum infiltration method shows 15% increment compared to roller infiltration method. The 60 ppi with roller infiltration method sponge shows 5 times increment compared to 30 ppi sponge with the same infiltration method. The same trend was observed for vacuum infiltration method for both size of sponges where 60 ppi sponge shows 7 times increment compared to 30 ppi sponge.

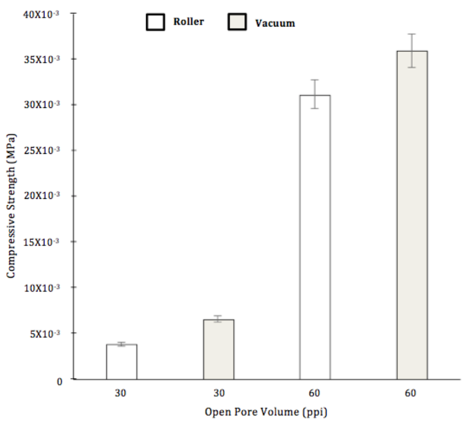


**Fig. 1.** *Compressive strength of HA scaffolds produced using different pore volume (ppi) template and different infiltration methods.*

The higher compressive strength of the sample fabricated by vacuum infiltration method may be due to the ability of slurry to infiltrate into the sponge with the help of vacuum applied during the fabrication method. Other than that, the vacuum environment allow the slurry to coat the sponge strut with formation of thick strut ceramic. The HA green body with a strong structures obtain via vacuum infiltration method will make a good densification of ceramic foam during sintering process.

When comparing the open pore size in polymeric template with the strength of sintered HA scaffolds, the 60 ppi foam has a higher compressive strength as compared to the scaffolds with 30 ppi foam. The 30 ppi foam has larger open pores inside the sponge.

Hence, during sintering process, the sponge will be burnt out and leave larger pores inside the ceramic foam. For 60 ppi foam, the densification of ceramic slurry during the sintering process is high since it contain small pores and thus high amount of ceramic slurry will flow into the sponge during the formation of green ceramic body. According to by Sooksaen & Karawatthanaworrakul [6], polyurethane template with larger average pore size contained less solid content which resulted in a decrease of compressive strength of the fired porous body. Larger average pore size contribute to the smaller ppi value for sponge template.

Fig. 2 shows the microstructure of polymeric foam and pores HA with closed and open cell on the porous HA prepared from 30 ppi template produced by roller infiltration method. The morphology of porous consists of open and interconnected pores, while the wall also looks exactly like the template. Ceramic foam consists of an assembly of irregularly shaped prismatic or polyhedral cells connected to each other with solid edge (opened cell) or faces (closed cell). At early stage of sintering, 400 to 500 ˚C, the organic compound and template will decompose and the densification process of ceramic particles occur. Gas was released during decomposition of polymeric sponge and contribute to the defect after sintering process [7].

Fig. 3 shows the microstructure of porous HA with different infiltration method at 30 and 60 ppi sponge. Based on microstructure, it is found that the infiltration method did not affect much on the microstructure of the HA scaffolds.

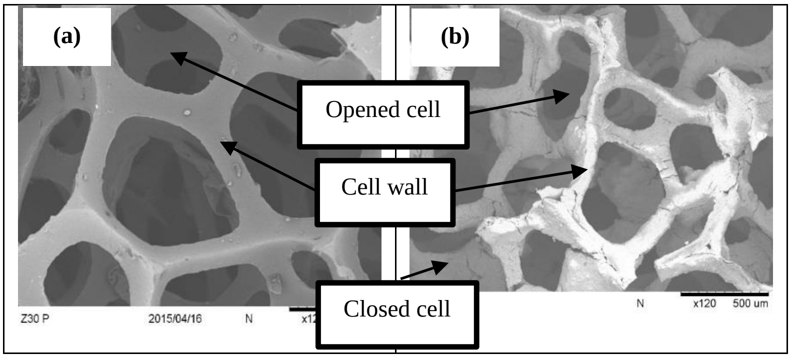


**Fig. 2.** Morphology comparison between (a) polymeric foam and (b) porous HA for 30 ppi template (Magnification of 120x)

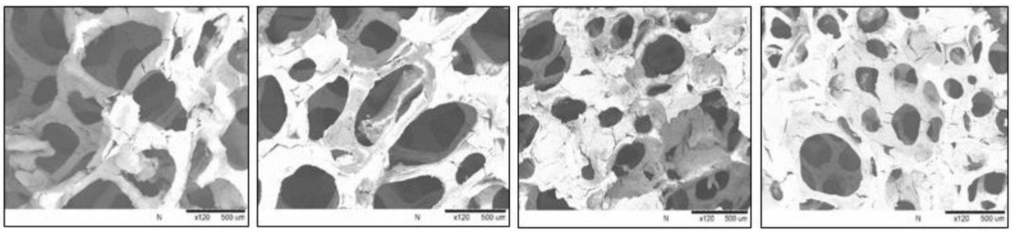

Fig. 3. Microstructure Microstructure of porous HA for each infiltration method and template pore size (a) roller with 30 ppi, (b) vacuum with 30 ppi (c) roller with 60 ppi and (d) vacuum with 60 ppi (Magnification of 120x)

Table 1 shows the average strut thickness and pore size for each sample with respect to the infiltration method and template open pore size. The average value of strut thickness and pore size were taken based on 25 readings. The effect of infiltration method and pore per inch of template can be clearly seen from the strut thickness that forms wall between the pores. The average strut thickness is the highest for roller infiltration method using 30 ppi template. In addition, the open pore size in starting sponge template influence the strut thickness. The 60 ppi template produce porous HA scaffolds with a low percentage of porosity with a formation of many small strut. The amount of strut also contribute to the formation of high density and low porosity porous HA. The average strut thickness for roller infiltration method with 30 ppi sponge shows the lowest compressive strength. This is because the template has large open pore size and the slurry did not completely filled the pores during the slurry impregnation process.

Although there are uneven distribution of pores in the HA scaffolds after sintering process, the compressive strength can be varied from one sample to another with different infiltration process. Other than that, the uneven distribution of pores is influenced by the template. The porous HA will mimic the template structure with the additional of binder as the materials to enhance the strength of unfired green body.

Besides that, porous HA scaffolds produced from roller infiltration method shows the highest average pore size compared to vacuum infiltration method. The ppi difference in the pore size may be influenced by the slurry impregnation process. In vacuum method, the slurry completely filled the open pores because of the low pressure in vacuum desiccator.

When comparing the effect of open pore size of template, it can be clearly observed that the 30 ppi sponge has the highest average pore size after sintering process. Pore size also influenced the strength of porous HA scaffolds. Smaller pore size will contribute to the high compressive strength of the porous network.

**Table 1.** *Average strut thickness and pore size for each sample with different infiltration method and template pore size.*

| Sample (method, template pore size) | Average strut thickness (μm) | Average pore size (μm) |
|---|---|---|
| Roller, 30 ppi | 103.3 | 313.3 |
| Roller, 60 ppi | 80.4 | 200.8 |
| Vacuum, 30 ppi | 105.7 | 301.1 |
| Vacuum, 60 ppi | 86.2 | 174.8 |

## Conclusions

Based on the SEM microstructure, it is found that microstructure of the HA scaffolds is not influenced by the infiltration methods. Porous HA scaffolds with vacuum infiltration method and 60 ppi sponge shows the highest compressive strength with moderate average strut thickness and lowest average pore size compared to those of samples produced by roller infiltration method at different template pore size.

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