# Pore Characterization of Porous Poly-Ɛ-aprolactone/Hydroxyapatite Nano Composite Analyse by Software Image J

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***Abstract*—**Supercritical $CO_2$ gas foaming method has been one of the favorable method in producing polymer composite for Bone Tissue Engineering due to absent of inorganic solvent that will might cause inflammation if in contact with cell tissue. Besides, it also gives the ability to control the process to obtain various pore size and porosity. This is the method to produce PCL/HA composites which is been recognize for its biocompatibility and mechanical structure. Several parameters of process such as foaming temperature and pressure will affect the pore characteristics alongside with the HA content. In this study, the effect of temperature, pressure and HA content will be investigated on the pore characteristic such as porosity, pore size, distribution and density. The samples were fabricated at 10MPa, 20MPa and 30MPa at 40°C and 45°C with 10%, 20% and 30% HA content. The characteristic are analyzed using Software Image J. Results show that increase in temperature and pressure cause the pore size, porosity and density to increase. Overall, the pores size obtained are in compliance with the requirement to be used in Bone Tissue Engineering.

***Keywords— PCL/HA composite blend, Supercritical $CO_2$, Pore Characterization, Tissue Engineering***

## I. INTRODUCTION

The usage of reconstruction procedures in orthopaedics has resulted a rise in surgical advancement and in the bone implant development. Synthetic porous scaffolds are used to be made of polymers, metals, ceramics or composite biomaterials. To design the ideal biomimetic artificial scaffold, a full understanding of the compositions, structures and biomechanical and biochemical properties of natural bone is required. The best scaffolds must exhibit the natural extracellular matrix (ECM) as much as possible. ECM in natural tissues supports proliferation, differentiation and cell attachment. These indicate that scaffolds shall contain a fitting biochemistry and nano/micro-scale surface topographies so that a complimentary binding sites can be achieved to regulate and control cell and tissue behaviour on full function, along with connecting to host cells. By having this feature, the growth of cells and new tissues can take place efficiently. It is also the growth factors carrier [13].

Polycaprolactone (PCL) is preferred since it is highly hydrophobic, has longer degradation times and highly biocompatibility [5]. PCL is a biodegradable polymer that has been recognized as a bone tissue compatible material without producing toxic response [9]. PCL has a melting temperature of 63°C [4]. But, PCL does not adhere to bone when are implanted alone because of lack of bioactivity and osteoconductivity. Hydroxyapatite (HA) accounts for about 65 wt% of bone which provides most of its strength and stiffness [2]. Thus, HA is needed to provide the osteogenic properties. Hydroxyapatite is biocompatible with bone and can be used for orthopaedic and dental implants. Therefore, by blending PCL and HA, a composite with better osteoconductivity, bioactivity and a more balance mechanical strength. Table 1 shows the criteria of an ideal scaffold for bone tissue engineering [12].

Table 1 Criteria and Requirement of Scaffold

| Criteria | Requirement |
|---|---|
| Biocompatibility | Support cells' attachment, and initiate tissue regeneration |
| Osteoconductivity | Encourage host bone adherence and growth into the scaffold |
| Biodegradability | Be able to degrade at a physiologically relevant rate |
| Mechanical properties | Maintain proper mechanical stability for tissue regeneration |
| Porous structure | High porosity (>90 %)<br>Pore diameters between 300 and 500 μm. |

Characteristics such as high porosity and an interconnected 3D macroporous network is required for cell proliferation, and cell adhesion is better with high surface area. All of these specifications shows optimal balance between materials and processing technique must be achieved so that the ideal morphological, function and structure are obtained for specific application. Various ways are out there in producing scaffolds. It is either by conventional techniques for example solvent casting, foaming, freeze drying and physical separation or advanced processing such as rapid prototyping [7]. Supercritical CO2 foaming has grab the attention in tissue engineering because of the allowance for fabrication of porous scaffold without the usage of organic solvent that might be bad for cells and tissue. Besides, the quite low pressure and temperature gives the possibility of processing biomaterials, cells and growth factors, aiming to produce bioactive porous scaffolds in a one step process [8]. scCO2 technology is already well known to be able to produce a wide range of biocompatible polymers with controllable morphology, purity and surface properties [7]. [10] states that by supercritical gas foaming method, scaffolds produced can reach up to 93% porosity and pore sizes be up to 100 mm. Strength obtained greater than 10 MPa, is the ideal mechanical requirements for tissue engineering. A macroporosity is necessary to enhance three-dimensional adhesion, proliferation and migration of cells, whereas microporosity channel with pore sizes ranging from 1 to 50microns is for nutrient and metabolic waste transport [9].

This method involves various temperature and pressure in producing the composite. Thermal history and the depressurization profile has effects on the morphology of the scaffolds pore. Since, particle size and morphology are two of the most vital properties that affect the behavior and stability, advance microscopic technique like Scanning Electron Microscopy (SEM) are used to analyze the morphology of the composite. SEM is the technique of choice for the evaluation of the scaffold morphology. SEM is capable to give the precise assessment of the morphology, size and

surface of particles. It just that it can't be used to evaluate pore distribution. Thus, pore distribution will be analyzed using software Image J. Other than process temperature and pressure, the HA content also has effects on the pore characteristic. So, this paper will cover the effect of temperature, pressure and HA content on the pore characterization of porous PCL/HA nano-composites.

## II. METHODOLOGY

### A. *Materials*

Porous PCL/HA composite has been prepared by foaming process at different temperature which are 40°C and 45° and pressure at 10, 20 and 30 MPa. The content of PCL/HA mixture is shown in Table 1.

Table 2: HA Content of PCL/HA Composite

| Design | PCL (wt%) | HA (wt%) |
|---|---|---|
| PCL | 100 | 0 |
| 10 N | 90 | 10 |
| 20 N | 80 | 20 |
| 30 N | 70 | 30 |

### B. *Morphology of PCL/HA composite blends*

The morphology of PCL/HA composite blend samples were assessed by field emission scanning electron microscopy (FESEM). The samples were cross sectioned, gold coated and analysed by FE-SEM (GEMINI: ZEISS SUPRA 55VP) under secondary electron imaging [1].

### C. *Pore characterization analysis*

The pore characterizations are analyzed by using Software Image J. The image obtained from FE-SEM are analyzed for their characteristics. One hundred pores for each sample were analyzed by using the "particle analysis" tools of the Image J software that enable to assess the area of each pores. Properties such as pore diameter, pore distribution, area, no of pores are all obtained from the analysis.

### D. *Porosity and cell density*

Porosity and density is a major properties in determining the suitability of the composite to be used in bone defect application. This also shows the effect of the parameters have on the scaffold characteristic. Equation 1 and 2 are the equations used to calculate the porosity and pore density.

$$Porosity = \left[1 - \left(\frac{\rho foam}{\rho composite}\right)\right] \times 100\% \qquad \text{Equation 1}$$

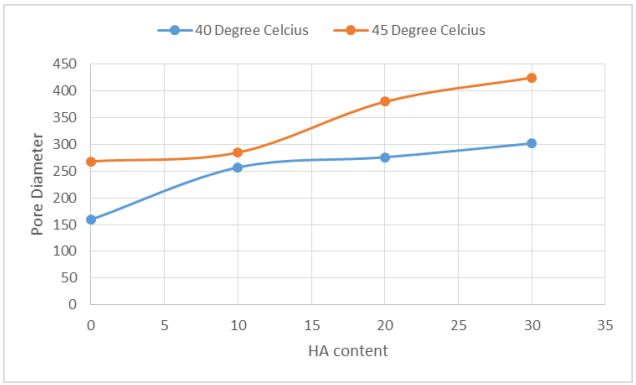
$$Cell\ Density = \left(\frac{nM^2}{A}\right)^{\wedge}\frac{3}{2} \qquad \text{Equation 2}$$

n = No of pores
M = FE-SEM Image Magnification
A = Total fesem area (cm²)

## III. RESULTS AND DISCUSSION

### A. *The effects of temperature on pore characterization*

Temperature and pressure define the amount of $CO_2$ solubilised within the material which has a major control on pores nucleation and growth. The foaming temperature also affects the width of the pore size distribution. In this study, the operating temperatures 40°C and 45°C. The effect of temperature at pressure of 10MPa with different HA content was shown in Figure 1.

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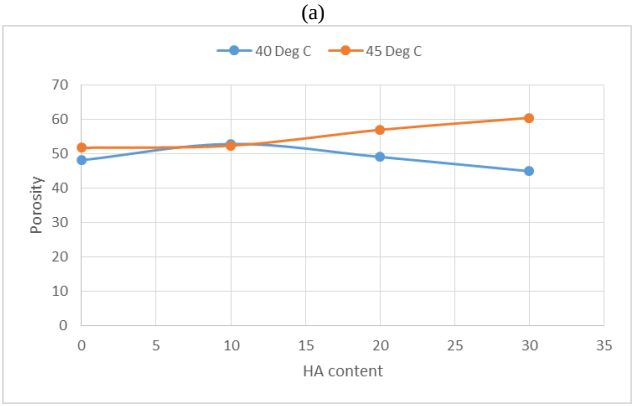


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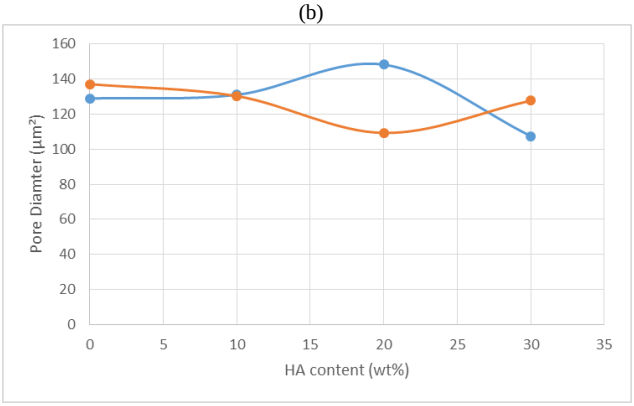


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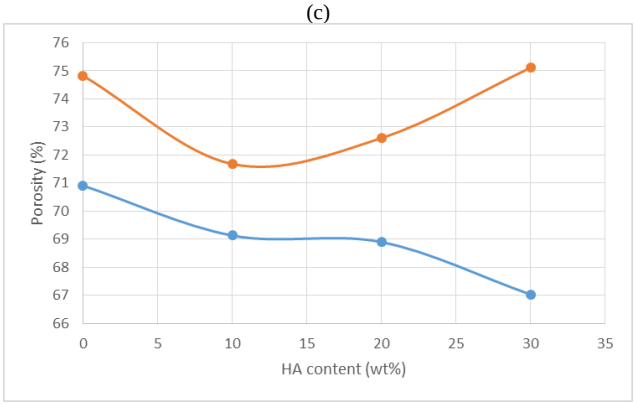


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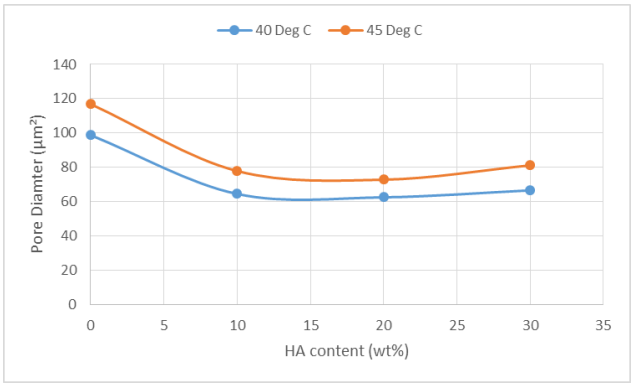
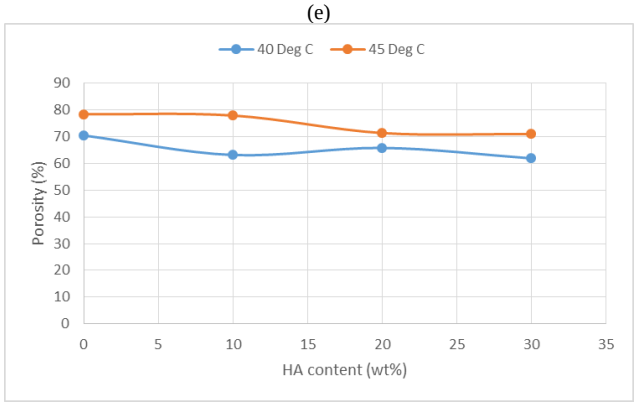



(e)



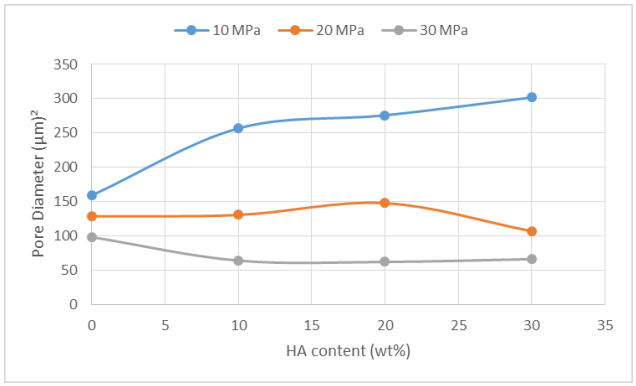

(f)

Fig. 1: The effect of temperature on (a,c,e) pore diameter and (b,d,f) porosity with HA content of 0, 10N, 20N and 30N at (a,b) 10MPa, (c,d) 20MPa and (e,f) 20MPa pressure

It can be seen that the pore diameter and porosity are both higher at 45°C process temperature. This is due to enlargement of pores when temperature are increased. The increase of temperature will reduce the solubility of $CO_2$ in the polymer because the $CO_2$ density will also decreased. Thus, the nucleation sites formed are fewer at high temperature. Higher temperature promote the diffusivity of CO2, resulting in better pore growth. Thus, formation of large pores occurred at higher temperature compared to lower temperature. The reduction of melting temperature due to the presence of sc-CO2 lower the viscosity of PCL when temperature is over 40°C. This results in coalescence and growth of pores contribute to wider pore distribution. Porosity under 40°C is also lower due to insufficient melting under that temperature conditions [3]. Even though the value are quite similar, still the porosity and pore diameter of the scaffolds are higher and larger at 45°C compared to 40°C.

## *B. The effects of pressure on pore characterization*

The results shown in Fig. 2 shows that as the foaming pressure increases the pore diameters decrease. By raising the foaming pressure, the dissolution of $CO_2$ in PCL becomes linear relationship, which leads to a high super-saturation level during initial stage of depressurization. This contributes to a higher nucleation density. $CO_2$ was consumed more in nucleation rather than pore growth, thus the smaller pore sizes. Porosity increases as the pressure increases, however there's not much different between 20MPa and 30MPa. Porosity are all around 80% when the pressure are raised to 20MPa for both temperature. Notice that porosity at 30MPa is slightly lower than 20MPa, the factor might be due to the collapse of pores at depressurization because of the saturation of polymer matrix by the superfluoussc-CO2 [3].

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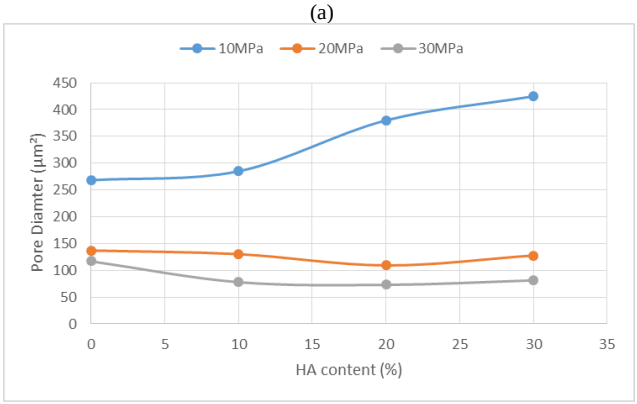


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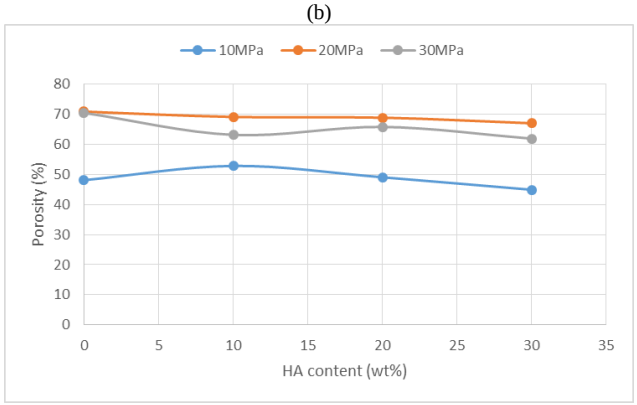


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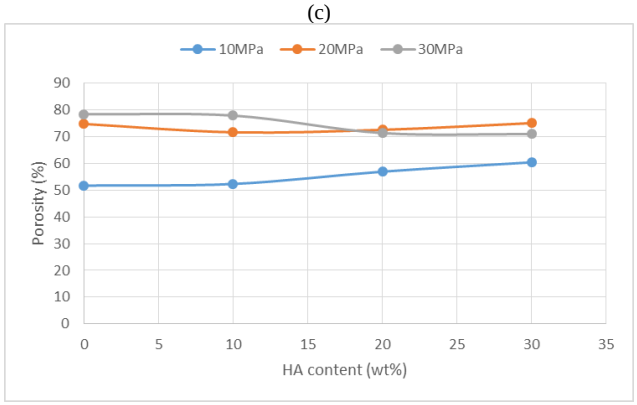
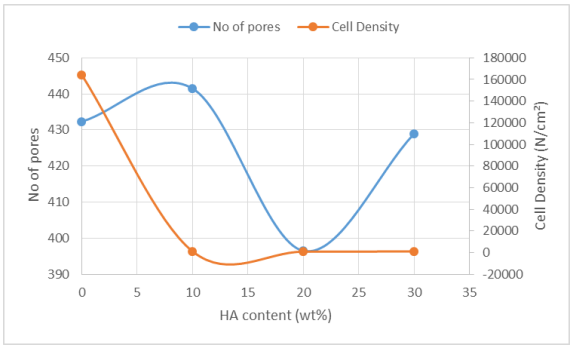



(d)

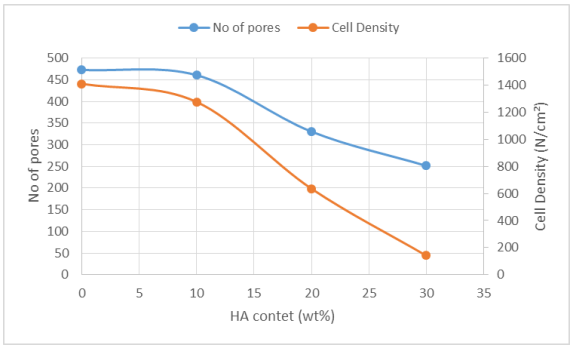
Fig. 2: The effect of pressure on (a,b) pore diameter and (c,d) porosity with HA content of 0, 10N, 20N and 30N at (a,c) 40°C and (b,d) 45°C

## *C. The effects of HA content on pore characterization*

The HA content in the PCL/HA composite has a major influent on the pore characteristic such as no of pores and pore density. HA that was blend with PCL might accumulate on PCL surface and thus affect the pore characteristic such as the density and no of pores.




(a)

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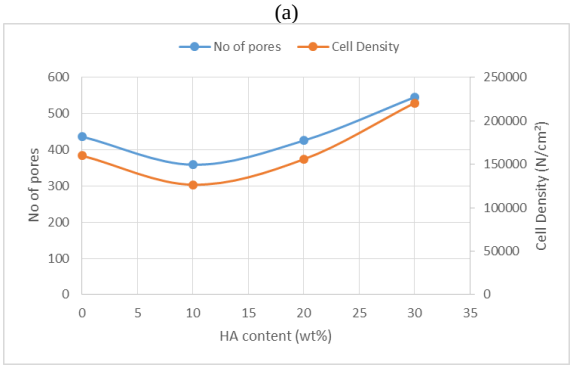


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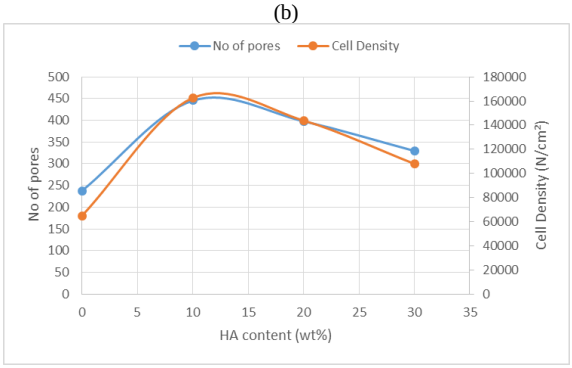


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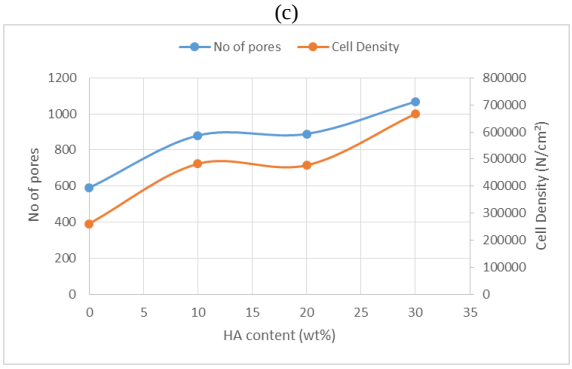


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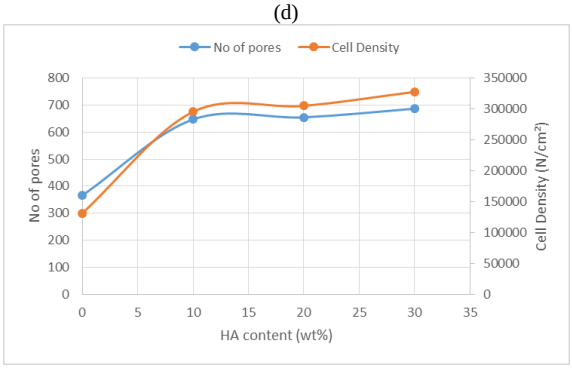


(f)

Fig. 3: The effect of HA content (PCL, 10 % HA, 20% HA and 30% HA) on the no of pores and pore density at (a) 10MPa 40°C (b) 10MPa 45°C (c) 20MPa 40°C (d) 20MPa 45°C (e) 30MPa 40°C and (f) 30MPa 45°C

From Fig. 3, it can be seen that the relation between no of pores and cell density is directly proportional. When the no of pores increase, the cell density also increase, except for Fig.3 (a), where for PCL (0%HA) the magnification of the image from FE-SEM is different than 10%, 20% and 30% HA content, thus different behavior for cell density is shown. At 40°C, for 10MPa, the highest no of pores is at 10% HA content. While for 20MPa and 30MPa, it is at 30% HA content. This goes the same for cell density, except for 10MPa due to different image magnification. Process condition at 30MPa, 40°C and 30% HA, gives the highest no of pores which is 1068 while the lowest which is 238 is at 20MPa, 45°C and 0% HA. Given the similar behavior of all the samples, indicates that it is a minor influence of HA content on the density and no of pores [8]. The pore density also increase as the pressure increase.

This is because if the pores are larger than there will be less pores over a certain area and therefore a decrease in the pore density. With the increase in HA content, the pores become more rigid and not as smooth. This is to improve the interlock between the scaffold and bone. Addition of HA particles was found to alter the morphology [6].

| Pressure | 10MPa | | 20MPa | | 30MPa | |
|---|---|---|---|---|---|---|
| Temperature | 40°C | 45°C | 40°C | 45°C | 40°C | 45°C |
| Pore Diameter | 159.3706667 | 268.2565 | 128.936 | 137.118 | 98.8526667 | 116.908 |
| No. of Pores | 432 | 473 | 437 | 238 | 590 | 366 |
| Pore Density | 164318.0116 | 1411.006243 | 160013 | 64972 | 261248 | 131485.628 |
| Porosity | 48.12393661 | 51.74921891 | 71 | 75 | 70 | 78.4209387 |
| Pore Distribution | Bimodal | Monomodal | Monomodal | Bimodal | Monomodal | Monomodal |

(a)

| Pressure | 10MPa | | 20MPa | | 30MPa | |
|---|---|---|---|---|---|---|
| Temperature | 40°C | 45°C | 40°C | 45°C | 40°C | 45°C |
| Pore Diameter | 256.96 | 284.961 | 131.014 | 130.29 | 64.6186667 | 77.9796667 |
| No. of Pores | 442 | 461 | 359 | 446 | 881 | 647 |
| Pore Density | 1185.269986 | 1277.243713 | 126535 | 162598.083 | 482471 | 295446.645 |
| Porosity | 52.85783388 | 52.35453673 | 69 | 71.6822351 | 63 | 77.9388084 |
| Pore Distribution | Monomodal | Bimodal | Bimodal | Monomodal | Bimodal | Monomodal |

(b)

| Pressure | 10MPa | | 20MPa | | 30MPa | |
|---|---|---|---|---|---|---|
| Temperature | 40°C | 45°C | 40°C | 45°C | 40°C | 45°C |
| Pore Diameter | 275.979 | 379.87 | 148.250667 | 109.272 | 62.69433333 | 72.922 |
| No. of Pores | 397 | 331 | 426 | 398 | 889 | 655 |
| Pore Density | 975.2501449 | 635.8059499 | 155773 | 143844.24 | 477155 | 304956.2115 |
| Porosity | 49.07447828 | 56.95383425 | 69 | 72.607755 | 66 | 71.38549675 |
| Pore Distribution | Monomodal | Monomodal | Bimodal | Monomodal | Bimodal | Monomodal |

(c)

| Pressure | 10MPa | | 20MPa | | 30MPa | |
|---|---|---|---|---|---|---|
| Temperature | 40°C | 45°C | 40°C | 45°C | 40°C | 45°C |
| Pore Diameter | 302.03 | 424.8845 | 107.487667 | 127.724667 | 66.723 | 81.28666667 |
| No. of Pores | 429 | 252 | 545 | 330 | 1068 | 687 |
| Pore Density | 1091.472741 | 140.8739266 | 220581 | 107966.859 | 667411 | 327564.7334 |
| Porosity | 44.92315508 | 60.44817177 | 67 | 75.1168138 | 62 | 71.01996885 |
| Pore Distribution | Monomodal | Monomodal | Bimodal | Bimodal | Monomodal | Monomodal |

(d)

Fig. 4: Summary of the values of scaffold characteristic for different HA content (a) PCL, (b) 10%, (c) 20% and (d) 30%

Fig. 4 summarizes all the values for important criteria of the scaffold produced by $scCO_2$. From the fig, the distribution pattern of the composite can be seen. Monomodal distribution means that it is not uniform, whereas for bi-modal the distribution is uniform. The porosity range from 50%-80% and the pore size from 100µm to 500µm. PCL/HA scaffolds with bimodal pore size distributions that were characterized by a macro-porosity, with pore sizes in the range 100 to 300mm, and a micro-porosity with a mean pore size in the 50–70 mm range [12].

There are several literature investigations demonstrating that pores of 5 µm size are necessary for tissue vascularization, while pores of 5–15 µm are optimal for fibroblast growth, between 20–125 µm for regeneration of skin, and in the range of 100–350 µm for regeneration of bone tissue [11]. This shows that scaffold produced by gas foaming method can be applied in Bone Tissue Engineering.

## IV. Conclusion

In this study we investigated the solid-state scCO2 foaming parameters on PCL/HA nano-composite characteristics. The results demonstrated that the control of the thermal history and foaming parameters is essential to design foams with suitable pore structure to be used in bone defect application. Composite with optimum morphology, porosity and pore size distributions were achieved by selecting the right pressure and temperature for the process. It is clearly seen that as the temperature and pressure increase, the porosity and pore size also increase but only to certain extent only, then it stays constant. All of these results shows that the pore size obtained are all eligible to be used in bone tissue engineering.

## Acknowledgment

Thank you to my supervisor and Universiti Teknologi Mara.

## REFERENCES

Akhbar, S. (2018). Performance of Polycaprolactone / Hydroxyapatite ( PCL / HA ) Composite Blended by Ultrasound Assisted Melt Blending, *5*(5), 235–250.

Alobeedallah, H., Ellis, J. L., Rohanizadeh, R., Coster, H., & Dehghani, F. (2011). TEM - částice, *25*(3), 12–19. Retrieved from http://medind.nic.in/taa/t11/i1sji/taat11i1sjip12.pdf

Chen, C., Liu, Q., Xin, X., Guan, Y., & Yao, S. (2016). The Journal of Supercritical Fluids Pore formation of poly ( □ -caprolactone ) scaffolds with melting point reduction in supercritical CO 2 foaming. *The Journal of Supercritical Fluids*, *117*, 279–288. https://doi.org/10.1016/j.supflu.2016.07.006

Coimbra, P., Almeida, J. P., Santos, A. C., Pereira, S., Cunha, H. P., Oliveiros, B., & Capela, C. (n.d.). Characterization of poly ( caprolactone )/ hydroxyapatite composite scaffolds produced by a melt extrusion additive manufacturing technique, 131–138.

Corrales, P., Esteves, L., & Vick, R. (2014). NIH Public Access, *14*(1), 15–56.

Guarino, V., Taddei, P., Foggia, M. Di, Fagnano, C., Ciapetti, G., & Ambrosio, L. (2009). The Influence of Hydroxyapatite Particles on In Vitro Degradation Behavior of Poly ε-Caprolactone–Based Composite Scaffolds . *Tissue Engineering Part A*, *15*(11), 3655–3668. https://doi.org/10.1089/ten.tea.2008.0543

Pascual, C. D., & Subra-Paternault, P. (2015). *Supercritical Fluid Nanotechnology*. *Supercritical Fluid Nanotechnology*. https://doi.org/10.1201/b19242

Salerno, A, Maio, E. Di, Iannace, S., & Netti, P. A. (2011). The Journal of Supercritical Fluids Solid-state supercritical CO 2 foaming of PCL and PCL-HA nano-composite : Effect of composition , thermal history and foaming process on foam pore structure. *The Journal of Supercritical Fluids*, *58*(1), 158–167. https://doi.org/10.1016/j.supflu.2011.05.009

Salerno, Aurelio, Zeppetelli, S., Di Maio, E., Iannace, S., & Netti, P. A. (2011). Design of bimodal PCL and PCL-HA nanocomposite scaffolds by two step depressurization during solid-state supercritical CO 2 foaming. *Macromolecular Rapid Communications*, *32*(15), 1150–1156. https://doi.org/10.1002/marc.201100119

Salerno, Aurelio, Zeppetelli, S., Maio, E. Di, Iannace, S., Netti, P. A., Salerno, A., … Novel, P. A. N. (2012). Novel 3d Porous Multi-Phase Composite Scaffolds Based On Pcl , Thermoplastic Zein And Ha Prepared Via Supercritical Co Foaming For Bone Regeneration To cite this version : HAL Id : hal-00681632. *COMPOSITES SCIENCE AND TECHNOLOGY*. https://doi.org/10.1016/j.compscitech.2010.06.014

Structure, M. M., Mechanical, H., Kim, J., Shin, K., Koh, Y., Hah, M. J., … Kim, H. (n.d.). Production of Poly( ε -Caprolactone)/Hydroxyapatite Composite Scaffolds with a Tailored Macro/Micro-Porous Structure, High Mechanical Properties, and Excellent Bioactivity. https://doi.org/10.3390/ma10101123

Wenden, A. L. (1981). Design of bimodal PCL and HA. □□□□□ □ □□□ □□□□□□ □□□□, *3*(September), 96–101.

Wu, S., Liu, X., Yeung, K. W. K., Liu, C., & Yang, X. (2014). Biomimetic porous scaffolds for bone tissue engineering. *Materials Science & Engineering R*, *80*, 1–36. https://doi.org/10.1016/j.mser.2014.04.001