Effect of Hydroxyapatite (HA) Content to the Pore Characteristic of Porous Polycaprolactone/ Hydroxyapatite (PCL/HA) Composite Analyze by Software Image J

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***Abstract*— The objective of this study is to investigate the effect of the HA content to the pore characteristic of porous PCL/HA composite. To achieve this objective, the foaming condition which are temperature, pressure and depressurized rate are manipulated. The foaming process was performed in the 35, 40 and 45 ◦C temperature range and pressure 10, 20, 30 MPa in order to control the final morphology, porosity and pore structure of the composite foams. The depressurized rate and duration PCL/HA are constant at less than 1 min & 4 hour respectively. The result in this study found that present of HA content significantly affect the pore structure and characteristic.**

Keywords*— hydroxyapatide, supercritical CO2, homogenous, polycaprolactone, scaffold.

I. INTRODUCTION

The utilization of bone grafts is the way to recovery the skeletal fractures, break, subtitute and regenerated the lost bone. It already shown by the highest amount of bone graft process that already performed over the world. The common way and standard of these is the autograft, but it can lead to many difficulty or problem (complication) such as, scar, sick, feel pain, blood loss, infection and donor-site morbidity. The other option is allografts, but they lack the osteoactive capacity of autografts which has the risk to carrying infectious disease or immune rejection. The different methologies, for example the bone graft substitutes, have focused on improved the efficiency of bone grafts and growth factors to stimulate the development of cells. The ideal bone graft or sometimes called as scaffold come from the biomaterials that impersonate the structure and properties or composition of natural bone. The most common and popular of bone grafts is the autograft, this process it will take (transplant) the bone or any tissues from the body to another body of the same patient. About 2.2 million bone grafts used in orthopedic procedures average for every years come from the high incident that has happened (Annis, Mosher, & Roberts, 2009). There have three main procedure or process of bone grafts which are the autographs, allografts and bone graft substitutes (Brydone A, Meek D, 2010). The tissue regeneration limit of these bone grafts can be determined by their own properties of osteogenic, osteoconductive and osteoinductive potential. The osteogenic potential and the capability of a bone graft driven by cells which take part in hte bone formation, for example osteoblasts, mesenchymal stem cells (MSCs), and osteocytes. The term osteoconductive stand to the matrix which stimulates cells of the bone to grow on the surface of bone. The important is osteoinductive capacity which in bone graft its needed and act as bone healing. It is refers to the stimulation or development of MSCs cell to differentiate into preosteoblasts which the beginning process of bone-forming process..

II. METHODOLOGY

2.1 Material

PC(60,000 g/mol) in pellet form was purchased from Shenzhen Esun Industrial Co.,Ltd..Irregular shaped hydroxyapatite powder was synthesized from clamshell by using chemical precipitation method. The density of HA powder was 3.1991±-.-189 g/cm^3. The mean particle size distribution was 56.01±4.91 µm which was analysed using a Malvern Instrument Mastersizer 2000. Then, the molear ratio of calcium(Ca) and Phosphorus(P), Ca/P was 1.59±0.0419 respectively, determined by Energy- dispersive X-ray spectroscopy(EDS). PCL(60,000 g/mol)in pellet form was puschased from Shenzhen Esun Industrial Co., Ltd. Needle shaped hydroxyapatite(HAN) powder was purchased from Berkeley. Meanwhile irregular shaped hydroxyapatite(HAS) powder was synthesized from clamshell by using chemical precipitation method. The density of the PCL,HAN and HAS powder was determined by Micromeritics Pycnometer using helium gas. The powder mean particle size distribution was analysed using a Malvern Instrument Mastersizer 2000, Meanwhile, the molar ratio of calcium(Ca) and phosphorus(P), Ca/P was determined by Energy-dispersive X-ray spectroscopy(FESEM).

Table 2.1: Physical properties for PCL, HAN and HAS powder.

Properties	Material		
	PCL	HAN powder	HAS powder
Density (g/cm^3)	1.1548±0.0027	2.9657± 000365	3.1991± 0.0189
Mean particle size (µm)	In pellet form	30.90±0.28	56.01±4.91
Ca/P ratio	-	1.64±0.0047	1.59±0.0419

2.2 Method

Extrusion

Before compounding process, PCL was dried under vacuum at 38°C for a minimum 10 hours, while HA powder were dried at 120°C for 10 hours to avoid moisture-induced degradation reaction, HA loading was varied from 10 to 40 wt% respectively. Table 2.2 shows the designation of the composite blends of PCL and HA. PCL and HA powder were mixed in a shaker before undergoing melt blending in an extruder equipped with an ultrasonic placed at the end of die. Melt blending was conducted at a temperature profile 110,100,110,110 and 100 °C from hopper to nozzle with a screw rotation speed of 9rpm. Ultrasound was used at 100% cycle at 400 watt and 22 Hz. After the extrusion, the strands were cooled in a water bath, and consequently, pelletized. Then, the pelletized PCL/HA composite blend were compressed using a hot press at temperature and pressure of 110°C and 9000 psi, respectively. To produce disc-shaped sample that were 20mm in diameter and 2mm thickness.

Table 2.2 PCL/HA composition

Designation	Content of PCL (wt%)	Content of HA (wt%)
PCL	100	0
10N	90	10
10S	90	10
20S	80	20
30S	70	30
40S	60	40

Foaming process

Foaming process was conducted by using 316 stainless steel cylindrical high pressure vessel (200ml) where carbon dioxide (CO_2) contacted the sample in a single pass batch system. The vessel was mounted in a universal oven (XUO32). The desired temperature was achieved by coil heated oven. The disc-shaped sample of PCL/HA were placed in the vessel that was subsequently heated and filled with CO_2 up to the desired pressure using a high pressure pump (brand:SAPAREX). The sample are exposed to high pressure CO_2 to allow saturation of CO_2 in the sample. The vessel was maintained at constant different temperature (35, 40 and 45 °C) and constant different pressure (10, 20and 30 MPa). Thermodynamic instability is then created by rapidly releasing CO_2 gas from the composite system, followed hy nucleation and growth of gas bubble in the sample. The holding time was constant for 4 hours before depressurized to 0MPa. The depressurized time was less than 1 minute.

2.3. Foams Characterization

Morphological analysis

The morphology of these sample were assessed using a field emission scanning electron microscope (FESEM), (model GEMINI: ZEISS SUPRS 55VP) under secondary electron imaging. Prior to observation by FESEM, the sample were fractured after immersed in liquid nitrogen and then coated with iridium for 45s. The FESEM image were converted to binary images and then analyzed by software Image J to evaluate the pore distribution and the mean pore size of the scaffold. The mean pore size was calculated by image analysis according to ASTM D3576. At least 100 pores distribution across the micrograph were analyzed. Three micrographs selected for each scaffold formulation.

Porosity and pore density of foamed composite

Porosity is a measure of the total pores spaces in a sample. It is a fraction of the volume of pore over the total volume of sample as percentage unit. The porosity of the foamed composite was determine by using following equation 1.

$$\text{Porosity } \% = [\,1 - (\frac{\rho F}{\rho P})\,] \times 100$$

Where ρF and ρP are the apparent densities of the foamed composite and initial composite respectively determined by using densitometer. Meanwhile the pore density is the number of pores per cm^3 of the foamed sample. The pore density can be estimated by equation 2.

$$\text{Pore density, } Nf = (\frac{nM^2}{A})^{3/2}$$

III. RESULTS AND DISCUSSION

3.1 Effect of foaming to the pore morphology of PCL & PCL/HA composite.

The pore morphology was investigate by using the scanning electron microscopy (SEM). Fig. 1 shows the effect of the temperature on the structure of the PCL samples treated at different temperature with the same enlargement which is 100 times. At the 45°C the pore formation was higher than at 40°C and the development of pore start at 35°C. Pore formation at 35°C was little compare to at 40°C and shows the development of microstructures in case of samples treated at different conditions of pressure temperature.

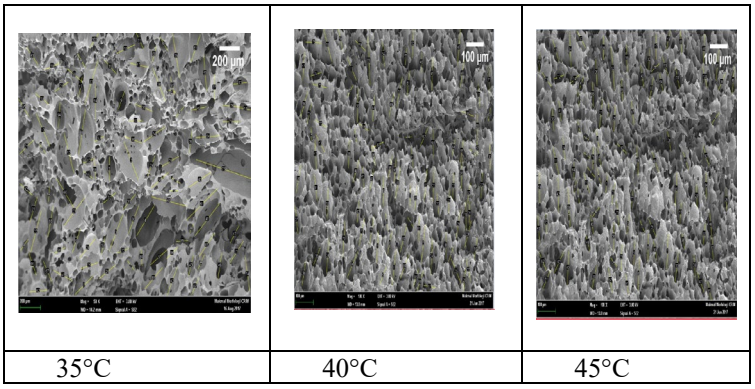

Figure 1: Effect of temperature on developed microstructure for PCL

When the temperature was increased from 35 °C to 45°C, there are certain aspect that need to be consider. The first one is viscosity of the sample at higher temperature is lower and it has less resistance for bubble to grow and coalesce. The increases of temperature will increase the size of the bubble. The rise of temperature in PCL sample at same pressure which 10MPa will increase the gas sorption due to the aggregate state of polymer change. Table 1 show the sample at 10MP of the average pore diameter, number of pore and porosity. At 45°C, the average pore diameter is increase compare to 40 °C and its follow to the requirement of pore morphology that need higher size of bone scaffold. At this point the availability of free volume within the system has increased and higher amounts of gas enter into the polymer. The average pore diameter increased whereas the cell density decrease with increasing the temperature. When foaming occurs at higher temperatures, the viscosity of the substrate is reduced and CO2 diffusivity increases, therefore favoring pore

growth. Also, as the temperature increases, the CO2 solubility in the polymer matrix decreases. At temperature 35°C is not suitable temperature for the foaming process which not good for the diffusivity which not cover the whole of the sample but only the part of sample. The number of pore also increase when the temperature increase .Its due to the higher diffusivity of CO2 which needed for the growth. The solubility and diffusivity of CO2 into polymers are important parameters that contribute to the understanding of the system behavior during processing and of the influence of process parameters on product properties. It describe the process that take place during gas foaming of PCL and the morphology of the final foam.

Table 1: Sample at pressure 10 MPa

Pore characterization	PCL	10S	20S	30S	40S
35°C					
Average pore diameter (µm)	157.96	Incompletely foaming	Incompletely foaming	Incompletely foaming	Incompletely foaming
Number of pore	122				
Porosity (%)	68.76				
Pore Distribution	Bimodal				
40°C					
Average pore diameter (µm)	72.62	70.72	65.39	130.44	156.48
Number of pore	248	264	310	232	302
Porosity (%)	68.76	69.72	66.69	65.85	51.94
Pore Distribution	Monomodal	Bimodal	Bimodal	Bimodal	Bimodal
45°C					
Average pore diameter (µm)	75.09	76.54	97.73	90.88	87.36
Number of pore	477	238	307	231	296
Porosity (%)	68.76	69.72	66.69	65.85	51.94
Pore Distribution	Monomodal	Monomodal	Monomodal	Monomodal	Monomodal

3.2 Pressure Effect to the Foam Morphology

The experiment with the high pressure are used in order to quantify the effect of the different pressure when the temperature constant which is at 45°C. The pressure increase from 10MPa to 30MPa will strongly influence to the objective of the analysis which are average pore diameter, number of pore and to the porosity. Positive effect of pressure increase on the pore morphology was the most noticeable in the lower pressure range, from 10 MPa to 20 MPa. The pressure increase from 10 MPa to 20 MPa resulted in creation of the foam with higher average pore diameter and more homogenous structure which can be seen in Table 2. The PCL foams obtained at 20 MPa and higher pressures had a homogeneous pore distribution, porosity about 65 until 69% and increases in average pore diameter which is in accordance with the requirements for scaffolds to be used in tissue engineering for proper bone ingrowth and vascularization. The average pore diameter at 30 MPa decrease in term of size compare to the 20 MPa. At higher pressures and high saturation of the polymer with gas, the excess of the filler particles or their weak interaction with polymer matrix. It also along with the increased contribution of hydrostatic pressure which are free volume in the polymer is decreased could be an additional obstacle to the mass transfer.

Table 2: Sample at temperature 45°C with different pressure

Pore characterization	PCL	10S	20S	30S	40S
10 MPa					
Average pore diameter (µm)	75.09	76.54	97.73	90.88	87.36
Number of pore	277	238	307	231	296
Porosity (%)	68.76	69.72	66.69	65.85	51.94
Pore Distribution	Bimodal	Bimodal	Bimodal	Bimodal	Bimodal
20 MPa					
Average pore diameter (µm)	146.50	146.50	183.99	175.67	153.64
Number of pore	351	318	417	314	238
Porosity (%)	69.35	65.15	67.50	67.17	68.14
Pore Distribution	Monomodal	Bimodal	Bimodal	Bimodal	Bimodal
30 MPa					
Average pore diameter (µm)	68.24	66.90	61.43	65.25	64.24
Number of pore	173	136	103	133	141
Porosity (%)	69.35	65.15	67.50	67.17	68.14
Pore Distribution	Monomodal	Monomodal	Monomodal	Monomodal	Monomodal

The number of pore are increase with the increase of average size diameter. At 30MPa number of pore is decrease is due to the collapse of the pore and must be homogenous. Higher solubility at lower pressure might be due to the lower degree of composite. At higher pressure the hydrostatic pressure contributes to a slower mass transport along with increased amount of HA particles that act as additional obstructions, and mobility. In order to explain and quantify the effect of scCO2 solubility on the thermal behavior. Porosity of the obtained foams in this study was in the range of 51–70%. Salerno et al. [14] have recently reported similar porosities of PCL and PCL-HA foams with 5–10%of HA micro- and nanoparticles (77–81%) obtained by foaming at 20 MPa and 40–45◦C.

Pressure increase up to 20 MPa had a positive effect on the diffusion of scCO2 in PCL. The variation of the diffusion coefficient is affected by the hydrostatic effect of pressure. During scCO2sorption, the molecules in the polymer–gas system are rearranged toward a new equilibrium conformation. At lower pressures, i.e. in the presence of a smaller amount of scCO2in polymer, the effect of scCO2is reflected in a higher mobility of the polymer chains which results in increased values of the diffusion coefficient. However, at high pressures, when there is a higher concentration of scCO2in the polymer, the hydrostatic pressure may play an important role and leading to decreased diffusivity.

Therefore, temperature and pressure influence foam morphology through their effect on gas solubility in the polymer and on viscosity of the substrate. The number of pores and their size are determined by the competition between cell nucleation and growth rates. At higher gas concentrations, nucleation dominates, and more pores with smaller diameter are expected. The opposite holds for smaller gas concentrations. The influence of CO2 solubility on the pore diameter of PCL foams.

3.3 The Effect of the Particle Size to Pore Morphology

The effect to the pore morphology examined for an optimal scaffold in bone tissue engineering. Embedding of HA particles in the scaffold resulted in rough surfaces on the composites. Instron actuator testing indicated that the tensile strengths and Young's moduli of scaffolds were influenced by both the porosities and pore sizes of the scaffold. It was apparent that increasing the concentration of porogen compromised the mechanical properties; and a larger porogen particle size led to increased tensile strength but a reduction in Young's modulus.

Table 3: Sample at pressure 10MPa with different HA content

Pressure (MPa)	10MPa	
Concentration	10s	10N
Pore diameter (µm)	65.473	70.999
Number of pore (n)	264	282
porosity	65.15	66.95

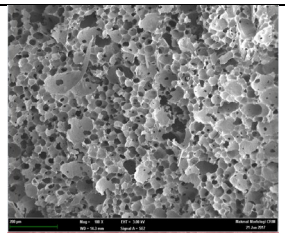	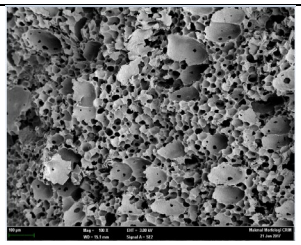
10 MPa 10s 40°C	10MPa 10N 40°C

Figure 3: Pore structure at 40°C with different HA content

Table 3 shows the different type of HA particle at the constant temperature which at 10MPa. The result show no significant different in term of the pore shape, number of pore and the porosity. The needle shape and irregular shape is not big effect to the result of morphology which only different at unit only. The needle shape use nanometer size and the irregular shape used micrometer size. Figure 3 shows the pore structure at constant temperature and pressure which not much different. Therefore, temperature and pressure influence foam morphology through their effect on gas solubility in the polymer and on viscosity of the substrate. The number of pores and their size are determined by the competition between cell nucleation and growth rates.

IV CONCLUSION

In this study the effect of the supercritical CO2 in processing PCL-HA content has been analyzed by using the software image J from the FESEM image. Besides, CO2 sorption in PCL increases steadily with pressure whereas the diffusivity is merely a function of temperature. Changes in morphology and occur as a consequence of the temperature and pressure changes. The objective of obtaining a homogeneous foam of bubble sizes in the range of several hundreds of microns is best obtained decompressing relatively slowly from conditions. .A high solubility of scCO2 in PCL-HA and its effected from the average pore diameter and the number of pore. Supercritical CO2 was then used for foaming poly (ε-caprolactone), and the connection between the process parameters (temperature, pressure), gas solubility in polymer, and foam morphology was studied.

V. RECOMMENDATION

In order to verify if these types of scaffolds would be a viable option or if the optimal composition has not yet been reached, more testing would be necessary. First of all, it would be beneficial to continue with some new dynamic mechanical analysis (DMA) tests, such as a stress relaxation test or frequency sweep to determine the materials' response to a constant strain or varying frequencies. Further, the effect of HA content on the viscosity of the materials could be measured using a rheometer. From the analysis, PCL/HA was desired in most aspects except when it came to modulus, which ultimately, was most important. However, there were certain ratios tested in this study and it is definitely possible that there is an optimal ratio out there that may be able to have maximal modulus while maintaining all the pore characteristics. In order to find this ratio it would be suggested testing some new wt % of HA content in PCL scaffolds.

VI. ACKNOWLEDGMENT

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