The Influence of Thermoplastic Zein (PEG, GLY and Zein) in Bio Composite PCL/TZ and HAp via Solid State Supercritical CO_2 Foaming

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Abstract—**This research is to study the characterization of the blended poly (ε-caprolactone) (PCL), hydroxyapatite (HA) and thermoplastic zein, (thermoplastic zein developed by mixing zein with glycerol (GLY) and poly (ethylene glycol) (PEG)) to form biodegradable porous structure and achieved via the solid state supercritical carbon dioxide ($scCO_2$) foaming technology. Characterization of the zein which is hydrophobic has been improved by mixing GLY and PEG to increase strength of zein contrast with the mixing of GLY and zein only which lead to a brittle structure. The plasticizers should have polar functional groups for effective zein plasticization, and a proper balance between polar and non-polar functional groups which important for plasticization efficiency. In this research area, this work reported on novel thermoplastic zein by mixing zein with (GLY and PEG) which might be a better way to synergistically improve the properties of natural polymer for foaming application. Thus, the effect of biocomposite material consist of PCL/HAp and TZ with different composition are investigated to generate porous structure via supercritical CO_2.**

In this work, reported the process of foaming with CO_2 take place for 6h at 50˚C and pressure about 20MPa with high depressurization rate. Foams were characterized by scanning electron microscopy. Result indicated that after saturation of the polymer material with CO2, high depressurization causes the thermodynamic instability and results in the formation of nucleated gas cells that give rise to pores within the scaffold. The blended composition of $PCL_{60}/TZ_{20}/HAp_{20}$ show result of well interconnected porous structure compared to other bio composite material prepared. Overall results show foaming effect produce foams with heterogeneous morphologies on bio composite material at relatively low temperature.

Keywords— **Bio composite, super critical carbon dioxide, thermoplastic zein, poly (ethylene glycol)**

I. INTRODUCTION

In forming an ideal bone scaffold, several biodegradable materials which is ceramics have been researched for bone repair and regeneration. Hydroxyapatite (HAp) is a bioactive ceramic where it is a major class of biomaterial for bone repair [1].HAp consist of criteria such as biocompatible, osteoconductive and non- toxic where these prove that it has identical biological behavior, chemical configuration and structure to native bone [2]. Limitation of HAp during it application are inherits brittleness, low mechanical stability which refuse it to use in large bone tissue regeneration and difficulty of shaping [1]. Therefore, weakness of HAp is overcome by adding synthetic polymers as inorganic filler to structure composite materials [2]. In order to satisfy the numerous requirements for scaffold materials using only a single material is not possible. Therefore, combination of synthetic polymer with HA has been reported to be a suitable way to yield porous designation that applicable for bone tissue engineering [3]. The blending of HA and synthetic polymer such as PCL cause a limitation, where the previous study claims that the presence of HA increased the density but had no significant effect on the porosity of the scaffolds [3].Therefore, approaches on the bio composite materials been studied to produce suitable materials to achieve better porosity for scaffold designation. Previous researcher has study the composite material by blending synthetic and natural polymers in order to defeat these drawbacks. Among the synthetic polymer materials which have been used for fabrication in bone tissue engineering scaffolds, PCL has been widely because of its biocompatibility, suitable mechanical properties, easy processing ability and non-toxic degradation products [4]. In literature has studied on blending of PCL with thermoplastic zein (TZ), a thermoplastic material developed by mixing zein with glycerol (GLY) [5]. Besides, HA particles in different concentration of 10wt% and 20wt% were added to the PCL-TZ blend for the preparation of multiphase composite [5]. However, our main focus here is to improve the zein characteristic because zein films prepared without plasticizers are brittle. Zein is a major storage protein of corn. Apparently, plasticizers with different chemical structures and physicochemical properties will produce different effects on the thermoplastic material form and a combination of different plasticizers should be a superior way to further develop the properties of zein for practical application. Study has proved that zein films containing only glycerol as plasticizer were brittle for all concentration of glycerol studied and all exhibited around 3% elongation [6]. In this research area, this work reported on created of novel thermoplastic zein by mixing zein with GLY and PEG in order to modified its supramolecular structure, decreased interaction between zein protein chains and also increase the mechanical properties of the zein film.

In a few years back, study on gas foaming process has widely known due to it suitability in the formation of tissue engineering scaffolds with fine controlled pore structures [7]. Generally, CO_2 is environmental friendly, non-flammable, and inexpensive while $scCO_2$ foaming can be applied to huge class of biodegradable polymers ranging from synthetic polyesters to natural materials such as thermoplastic proteins and saccharides as well as their combination[8]. Besides, temperature and pressure of scCO2 which is low (31.1˚C and 73.8MPa, respectively) create the material function to be design as drug delivery systems and bioactive engineering scaffolds. Along with this research line, the study is on the influence of TZ in PCL/HAp and TZ bio composite foaming via $scCO_2$.

II. METHODOLOGY

A. *Materials*

The following materials were used to prepare the biocomposite material of porous scaffold: Hydroxyapatite (HA), Polycaprolactone (PCL) and maize zein powder, were purchased by Innovative Pultration. Polyethylene glycol (PEG) 400 and Glycerol are used as plasticizer for the preparation of the TZ.

B Methods

Zein powder as received by supplier was pre-mixed with the plasticizers (PEG and GLY) in a beaker using spatula to provide a crude blend. Then, the blend was exposed to the temperature and shear stresses in a twin counter rotating internal mixer connected to a control unit for thermoplasticization. The mixing chamber (volume of $50cm^3$) was filled with 50 g total mass for the experiments. Plasticizers content used which is 25wt% out of it were 80% PEG and 20% GLY were used to mix with 75wt% zein to form thermoplastic zein. Mixing temperature, T_{mix}, speed of rotation, N and mixing time, t was 80ºC, 50 min^{-1} and 10 min.

In multiphase biomaterials preparation, PCL pellets was first melted at 70°C, 20 rpm for 2 min and, subsequently, TZ and/or HAp were added together to form melting process in twin counter rotating internal mixer and mixed at 70°C, 80 rpm for 10 minutes [7]. Table 1 shows four different compositions of the system prepared. Next, the bio composite materials that successfully formed were compressed moulded at 80°C and 30 MPa into 2 mm-thick plates by a hot press.

Foaming experiments were carried out on disc shape samples (d=10mm and h=2mm). Each samples were covered with cotton wool for smooth penetration of CO_2 on sample surfaces. The samples were solubilized with CO_2 for 6 hours at 50˚C and 20MPa [7, 9] and subsequently high depressurized the pressure to the atmosphere allowing samples foaming. The stabilization of the pore structure was achieved by cooled down the foam in the ambient temperature and removed from the vessel.

Table 1 Composition of the different systems prepared.

Biocomposite Formulation	PCL content (wt%0	TZ content (wt%)	HAp content (wt%)
PCL_{80}/HA_{20}	80	-	20
PCL_{50}/TZ_{30}-HA_{20}	50	30	20
PCL_{60}/TZ_{20}-HA_{20}	60	20	20
PCL_{70}/TZ_{10}-HA_{20}	70	10	20

C. Thermoplasticized Zein Characterization

Thermal degradation of the thermoplastic zein was investigated by Thermogravimetric (TGA) and differential scanning calorimetric (DSC) analyses. In particular, TGA experiments were carried out on a TGA 2950 (TA Instruments, USA) over a temperature range of 30°C to 600°C at 10°C/min under inert atmosphere. For DSC analyses (DSC Q1000, TA instruments, USA), samples with $40\mu L$ were prepared and carried out in temperature range from -80 °C to 300°C at 10°C/ min under inert atmosphere.

D. Foams characterization

The morphology of the foams was determined by scanning electron microscopy (SEM) were converted to binary images.

RESULTS AND DISCUSSION

B. Mixing of Thermoplastic Zein)

Preparation of thermoplastic zein (TZ) (zein 75wt% + PEG 80% + GLY 20%) show a well mixing and the sample produced do not lead to brittleness. Limited range of useful plasticizers for zein lead to the application of fatty acid and their derivatives glycerol and polyethylene glycol which claimed to be the most commonly used plasticizers in tissue engineering application [10]. Among the plasticizers that available the most effective blended with zein are Glycerol and PEG [11]. Plasticizers that are used may penetrate the protein networks, generate hydrogen bond with the protein molecules and eventually rising the splitting among the protein chain [12]. Previous reports shown that the plasticizing effect of PEG increased when the concentration increased and higher zein concentration also shows the effect such as turn into soft and sticky when blended with PEG [12]. Hence in agreement with these result, PEG concentration which is 80% is selected to be combined with zein. According to J.W.Lawton [13], amount of 20% GLY show the ability of GLY to plasticize zein protein contrast with increase of 30% of GLY which show no improvement on elongation of zein film. In agreement with these results, decision on 20 % concentration of GLY were used in mixing with zein to form TZ.

According to the study the effect of water vapour permeability (WVP) is depend on the concentration of plasticizers used [6]. The ratio of GLY to PEG was decreased lead to the decrease of WVP too. PEG is more hydropobic than GLY because of its functional groups, hence the study mention that WVP of zein film containing only GLY brought to higher result of WVP compare to an equal ratio (50:50) or one of the plasticizer was high in ratios, (0:100) for GLY/PEG [6]. According to Hansen's classification system, secondary plasticizers made up of glycerol contains hydrophilic molecule brought to limited effect on zein when used alone because of its incompatibility with zein protein that would lead to breaking phase and increased WVP [13, 14]. Since in this research work reported the ratio of GLY to PEG was 20:80 therefore, it is expected that WVP are lower than those for equal ratio of GLY/PEG zein film. Discuss on the mechanical properties of thermoplastic zein, it might be lot of improvement because of the combination of GLY and PEG as plasticizers. Based on the previous work reported that, GLY has been commonly used in protein based films as a plasticizer but it tends to migrate to the film surface [6]. The effect of elongation (E) on zein film when mixing with GLY/PEG has been reported by H.J Park [6] where they observed that E of zein films containing only glycerol was about 3% however addition of 0.39mL PEG /g protein to zein film increased by 94%. Addition of PEG in zein film tend to increase the E value. This is because zein have high hydrophobicity which can create interaction with PEG to received better linkages between the protein molecules.

Glass transition refers to the change from a hard, monocrystalline, glass-like material to rubbery solid, and the glass transition temperature (T_g) is the point at which this transition occurs with a change of heat capacity (C_p). In Fig.1 reported DSC result for thermoplasticized zein. DSC curve shows a glass transition (T_g) occur at 77.62˚C which due to the present of plasticizers form synergistic interaction between zein protein molecule. Present of glycerol in zein protein chains may build up the number of end groups that lead to reduction of T_g [15]. The result could be compared with previous literature where the glass transition is observed around 60˚C when the mixed plasticizer which was GLY/PEG equal in ratio of 1:1 [16]. Moreover, the DSC analyses of the pure zein revealed the presence of thermal transition at 161.88˚C for the pure protein which can be attributed to the T_g. This result was in agreement with those previously reported work where almost similar T_g value obtain by A.Salerno [17] for pure zein which is 162˚C. Hydrophobic interaction, van der Waals and hydrogen bonding are mainly give effects on the glass transition temperature of polymers that reflects the tightness of molecular packing or supramolecular structure.

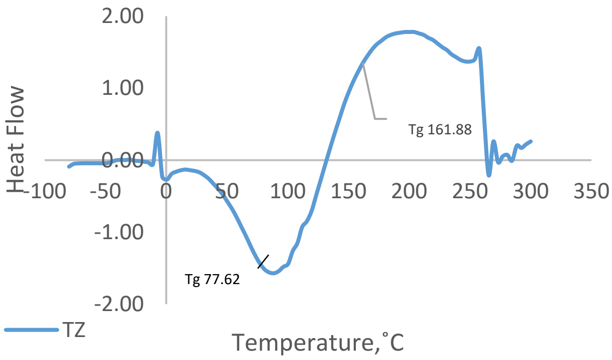

Fig 1. DSC curve of thermoplasticized zein

B. Thermal Properties

Figure 2 shows TGA curves, for different multiphase systems include those of neat PCL and thermoplastic zein. TGA curve of the TZ showed a weight loss in the range of 50 to 100 ˚C because of the evaporation of water and low molecular weight compound such as PEG molecules from the TZ phase while a steep weight decrease starts from 330 to 400 ˚C and can be related to the degradation of zein protein. However, for PCL curve showed the steep weight decrease starts from 400 to 450˚C due to the degradation take place. Blended of three different materials with different composition give significant effect based on the TGA curves plotted. PCL_{50}-TZ_{30}-HA_{20} and PCL_{70}-TZ_{10}-HA_{20} shows a less of weight loss which is about 5% at the temperature 410 until 600˚ C. However, PCL_{60}-TZ_{20}-HA_{20} shows slightly increase of weight loss which is estimated about 7% starting from the temperature of 400 to 460˚C. The difference in the weight losses are due to the different composition of TZ and PCL used in the system prepared instead of HAp which remains the same for overall composition. These results proved the heterogonous nature of the system prepared.

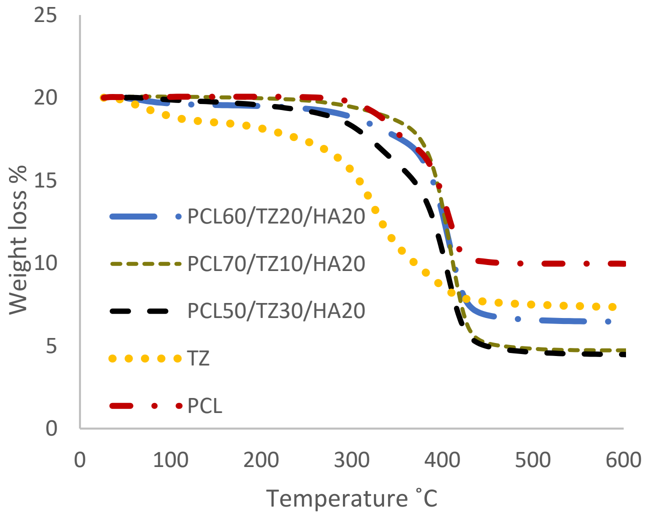

Fig 2. TGA curves of different multi-phase systems prepared. Neat PCL and TZ also reported for comparison.

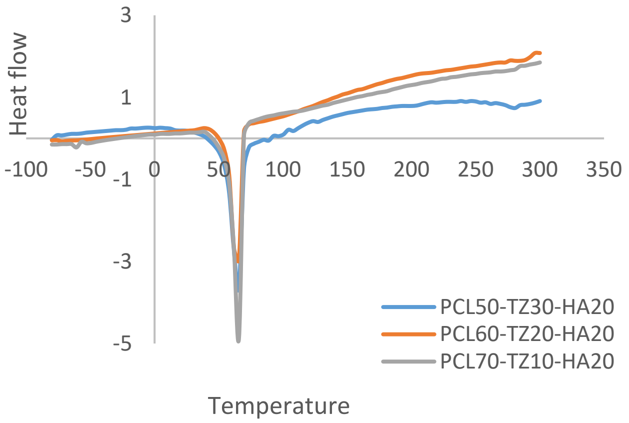

Fig 3. DSC curve of different multi-phase systems prepared for comparison.

In figure 3 shows the DSC curve of the three different multi-phase system to reveal the presence of thermal transition occur in the systems. DSC curve for PCL-TZ-HA showed a glass transition (Tg) appeared when the temperature around 50˚C. Polymer which undergoes glass transition cause the physical and chemical properties of the polymer change significantly. Results of the thermal analysis on the composition of PCL-TZ-HA provide useful information for the selection of processing temperatures in foaming (e.g. solubilization and foaming temperatures). The gas foaming temperature selected with the minimum temperature close to

Tg. A.Salerno [17] observed the effect at the temperature below the Tg of the Thermoplastic Gelatin (TG) cause the foaming is retarded due to the steep rigidity of the polymer or blowing agent solution. Glass transition is the phenomena of the amorphous polymers and amorphous portion of semi crystalline polymers. The endotherm curve appear on the DSC curve is mainly due to the melting of crystal. This peak revealed on the melting point of the polymer.

C. scCO$_2$ foaming of PCL/HA and PCL/TZ/HA$_{20}$

Super critical CO$_2$ foaming has a tendency to plasticize where polymeric biodegradable scaffold material, such as PCL at relatively low temperature, have brought to a new path for the design and fabrication of porous scaffold [18]. Selection of proper operating condition mainly blowing agent type (CO$_2$, N$_2$ and or their mixture), foaming temperature, TF, saturation pressure and pressure drop rate give broad effect on the pore structure features of porous materials prepared via scCO$_2$ foaming process.

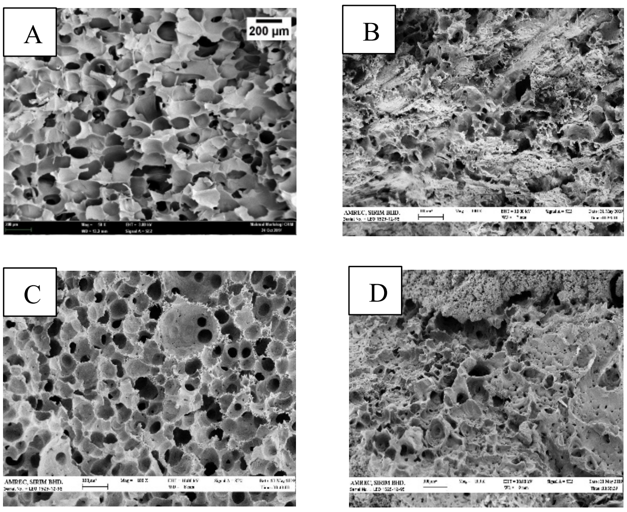

Fig 3. SEM micrograph images of the cross section of PCL/HAp and PCL/TZ/HAp foams: (A) PCL80/HAp20 (B)PCL70/TZ10/HAp20(C) PCL60/TZ20/HAp20 (D) PCL50/TZ30/HAp20

Fig.3 reported on the SEM morphology of the cross section of PCL80/HAp20 and PCL/TZ/HAp of different composition foams. As shown, all of the sample have been fixed their processing parameter such as saturation pressure equal to 20MPa, T_F 50˚C, foaming for 6hr and high depressurized. Selection on the foaming parameters process bring the target for perfect grow systems and was based on the previous work on the multiphase systems [5, 9].It clearly evidencing that the heterogeneous distribution on the size of the pores were promoted by the foaming process (Fig.3B,3C,3D). The foaming temperature selected which is 50˚C do not exceed the melting point of PCL plus blending with HA and TZ bring effect on pores generation when foaming with scCO$_2$. (Fig.3C). The supercritical CO$_2$ foaming of plastics requires that CO$_2$ diffuse into the material at supercritical conditions. According to the literature, result obtained at which lower temperature of foaming cause the TZ foams and indicate small cells separated from each other by thick walls, generate highly irregular and rough pore surfaces. [17]. Low T_F that been used in scCO2 were significantly lower than the TZ degradation temperature (323.9˚C) which preserve the chemical structure of the thermoplasticized protein. At a subsequent stage, a sudden release of pressure triggers CO$_2$ expansion, generating bubbles within the material. In particular, agreement with the results, evidence on similar multiphase system PCL/TZ/HA of increasing of pore size was observed after the increase of the depressurization time from 15 to 120s. [9]. The strong obvious interconnected of porous structure was built from the composition of PCL60/TZ20/HA20 (Fig.3C) comparing to the (Fig.3A) where the effect of foaming process on PCL80/HAp20 shows low interconnection. The observation on low interconnection may be ascribed to the effect of HAp particles distributed quite homogeneously throughout the mass of PCL matrix. The distribution of the HAp particles within the mass of the scaffolds is an important concern. Similar results were obtained from literature indicating a minor effect of the organic filler on the density and porosity of the foams PCL/HAp [19]. Although it is well known that the fusion of inorganic particles within a polymer may affects its foaming behavior, the observed effect may be analyzing by recognize the multi-phase system that been prepared. Fig. 3B and Fig.3C give clear SEM morphology of less porous distribution occur on the composition structure. This happens due to non-equilibrium diffusion of CO$_2$ on the surface of the material structure. Position of the material composition inside the vessel during conducting the scCO$_2$ play an important role in order to stabilize the diffusion of CO$_2$ within the surface materials.

III. CONCLUSION

The application of TZ with different formulated composition have been proof that porosity may occur when TZ blended with PCL/HAp via $scCO_2$ technology. This study has achieved the objectives and counter the problem statement of this research area.

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