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Influence of Thermoplastic Zein in PCL/TZ and HAp Bio Composite via Solid State Supercritical CO_2 Foaming

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ABSTRACT

This study is aimed to investigate the characteristics of the composite containing blended poly(ε-caprolactone) (PCL), hydroxyapatite (HA), and thermoplastic zein (TZ). Thermoplastic zein was developed by mixing zein with glycerol (GLY) and polyethylene glycol (PEG). The thermal properties of mixed TZ and bio composite were characterised to investigate the characterisation of PCL/TZ/HA composites. The bio composited was then molded, producing porous structure via solid state supercritical carbon dioxide ($scCO_2$) foaming process. The specimen was saturated with CO_2 for six hours at 50˚C and saturation pressure of 20MPa at a fast depressurisation rate. The morphology of the porous specimen produced was characterised using scanning electron microscopy (SEM). The results indicated that after polymer saturation with CO_2, high depressurisation causes the formation of nucleated gas cells that give rise to pores within the foamed specimens. The blended bio composite with the composition of $PCL_{60}/TZ_{20}/HAp_{20}$ exhibits a well interconnected porous structure compared to other bio composite prepared. The foaming effect produce foams with heterogeneous morphologies on bio composite material at relatively low temperature.

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Keywords: *thermoplastic zein, hydroxyapatite, porosity, foaming, supercritical carbon dioxide, poly (ethylene glycol)*

INTRODUCTION

Generally, materials that are commonly used for fabricating the bone tissue engineering scaffold can be classified into four groups, namely polymer, ceramic, metallic, and composite [1]. Synthetic polymer played a huge part in bone tissue engineering scaffold due to excellent mechanical properties, biocompatibility, biodegradability, and ease to fabricate into different shapes [2]. The most commonly utilised synthetic polymers are aliphatic polyesters such as poly (lactic acid) (PLA), poly (glycolic acid) (PGA), and polycaprolactone (PCL), and their copolymers. Meanwhile, ceramic is well understood on their promising properties, such as biocompatibility, osteoconductive (allows the bone cell to attach and encourages the growth of bone cell), osteoconductive (promotes the formation of new bone-cell), as well as their similarity with natural bone composition [3]. Numerous ceramics such as hydroxyapatite (HA), tricalcium phosphate, calcium silicate ($CaSio_3$) ceramic families, and bioactive glass are widely used for bone regeneration.

HA is a bioactive ceramic where it is a significant class of biomaterial for bone repair. HA is biocompatible, osteoconductive, and non- toxic material where proves that it has identical biological behaviour, chemical configuration, and structure to native bone [4]. Limitations of HA include brittleness and low mechanical stability, preventing it from being used in large bone tissue regeneration [5]. These shortcomings can be overcome by adding synthetic polymers such as inorganic filler to the HA based composite.

In recent years, the HA-based composite is preferable to satisfy the numerous requirements for scaffold materials compared to a single material. The combination of a synthetic polymer with HA has been reported to be a suitable way to yield porous designation that applicable to bone tissue engineering [6]. It is claimed that the blending of HA and a synthetic polymer such as PCL increased the density due to the inclusion of the former but had no significant effect on the porosity of the scaffolds [6]. Therefore, approaches were undertaken to produce suitable materials to achieve better porosity for scaffold designation. Salerno *et al.* [7] has studied composite material provided by blending synthetic and natural polymers, which is PCL and thermoplastic zein (TZ), to defeat these drawbacks. Besides, previous work has reported on the blending of PCL with TZ by mixing zein with glycerol (GLY), and HA affected the microstructural properties resulted in improved adhesion and osteogenic differentiation capability, thus demonstrating the potential for bone scaffold [9].

However, the main focus of the present work is to improve the zein characteristic by adding GLY and PEG. Zein is a major storage protein of corn. Apparently, plasticisers with different chemical structures will produce different effects on the thermoplastic material form. The study has proved that zein films containing only glycerol as a plasticiser were brittle for all concentrations of glycerol studied, and all exhibited around 3% elongation [6]. In this present work, a novel thermoplastic zein by mixing zein with GLY and PEG was produced. The blended of zein with GLY and PEG is to modify its supramolecular structure, reduce interaction between zein protein chains, and to increase the mechanical properties of the zein film.

A few years back, a study on the gas foaming process has widely known due to its suitability in the formation of scaffolds with fine controlled pore structures [7]. Generally, CO_2 is environmentally friendly, non-flammable, and inexpensive, while $scCO_2$ foaming can be applied to biodegradable polymers ranging from synthetic polyesters to natural materials [10]. Besides, temperature and pressure of $scCO_2$, which is low (31.1˚C and 73.8MPa, respectively), create the material function to be designed as drug delivery systems and bioactive engineering scaffolds. Along with this research line, the study is aimed to determine the influence of TZ in PCL/HAp and TZ bio composite foaming via $scCO_2$.

METHODOLOGY

Materials

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

Mixing Process

Zein powder, as received by the supplier was pre-mixed with the plasticisers (PEG and GLY) in a beaker and mixed in a twin counter-rotating internal mixer (Rheomix 600 Haake, Germany) connected to a control unit (Rheocord 9000 Haake, Germany) for thermoplasticisation. The mixing chamber (volume of 50 cm3) was filled with 50 g total mass for the experiments. Plasticiser content used, which is 25wt%, out of it were 80% PEG and 20% GLY was used to mix with 75wt% zein to form thermoplastic zein. The mixing temperature, T_{mix}, speed of rotation, N was adopted, and mixing time, t was 80 °C, 50 min^{-1}, and ten min, respectively.

In multiphase biomaterial preparation, PCL pellets were first melted at 70 °C, 20 rpm for two minutes (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 ten min [7]. Table 1 shows three different compositions of the system prepared with increased the composition of TZ in bio composite materials. Next, the bio composite materials that successfully formed were compressed molded at 80 °C and 10 MPa into 2 mm-thick plates by a hot press.

Table 1: Composition of a Different Specimen Prepared

Sample	PCL (wt%)	TZ (wt%)	HA (wt%)
PCL_{70}-TZ_{10}-HA_{20}	70	10	20
PCL_{60}-TZ_{20}-HA_{20}	60	20	20
PCL_{50}-TZ_{30}-HA_{20}	50	30	20

Foaming Process

Foaming experiments were carried out on disc shape samples (dimension = 20mm and thickness = 2mm). Each sample was covered with cotton wool for smooth penetration of CO_2 on sample surfaces. The samples were solubilised with CO_2 for six hours (h) at 50 ˚C and 20 MPa [7, 11], and subsequently, high depressurised pressure to the atmosphere was applied, allowing sample foaming. The stabilisation of the pore structure was achieved by cooling down the foam in the ambient temperature and removed from the vessel. The morphology of the foamed specimens was assessed using a Scanning Electron Microscopy (SEM) image (LEICA mod S440).

Thermoplastic Zein Characteristics

Thermal degradation of the thermoplastic zein was investigated using 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 an inert atmosphere. For DSC analyses (DSC Q1000, TA instruments, USA), samples with 20 mg were prepared and carried out in temperature range from -80 °C to 300 °C at 10 °C/ min under an inert atmosphere.

RESULTS AND DISCUSSION

Mixing of Feedstock

Preparation of thermoplastic zein (TZ) (zein 75wt% + PEG 80% + GLY 20%) shows a well mixing, and the samples produced are not brittle. Limited range of useful plasticisers for zein leads to the application of fatty acid and their derivatives glycerol and polyethylene glycol, which claimed to be the most commonly used plasticisers in tissue engineering applications [10]. On top of that, glycerol and polyethylene glycol are the most effective plasticisers blended with zein. Plasticisers that are used may penetrate the protein networks, generate hydrogen bonds with the protein molecules, and eventually raise the splitting among the protein chain [13]. Tillekeratne *et al.* [13] reported that as the PEG concentration increased, the plasticising effect was improved significantly, which zein turn into soft and sticky. Hence in agreement with these results, 80% PEG concentration is selected to be combined with zein. According to Lawton [14], 20% GLY gave the optimum effect of plasticising zein, whereas increased up to 30% of GLY shown no improvement in the elongation of zein film. In agreement with these results, a decision on 20 % concentration of GLY was used in mixing with zein to form TZ.

Thermal Properties

Glass transition refers to the change from a hard, monocrystalline, glass-like material to rubbery solid. The glass transition temperature (Tg) is the point at which this transition will create a change in the properties of the material, the polymer chains reorient, requiring a change in energy to heat. This shows as an inflection on the curve. Figure 1 shows the DSC result for thermoplastic zein. DSC curve shows a glass transition (Tg) that occurred at 77.62 ˚C due to the presence of plasticisers form synergistic interaction between the zein protein molecule. The inclusion of glycerol in zein protein chains may build up the number of end groups that lead to the reduction of Tg [15]. Huo *et al.* (2018) reported that the glass transition was observed at 60 ^{o}C for GLY/PEG composite [16].

Moreover, the DSC analyses of the pure zein revealed the occurrence presence of thermal transition at 161.88 ˚C for the pure protein, which can be attributed to the Tg. This result was in agreement with those previously reported work was almost similar Tg value obtained by Salerno [17] for pure zein, which is 162 ˚C. Hydrophobic interaction, van der Waals and hydrogen bonding are mainly giving effects on the glass transition temperature of polymers that reflects the tightness of molecular packing or supramolecular structure.

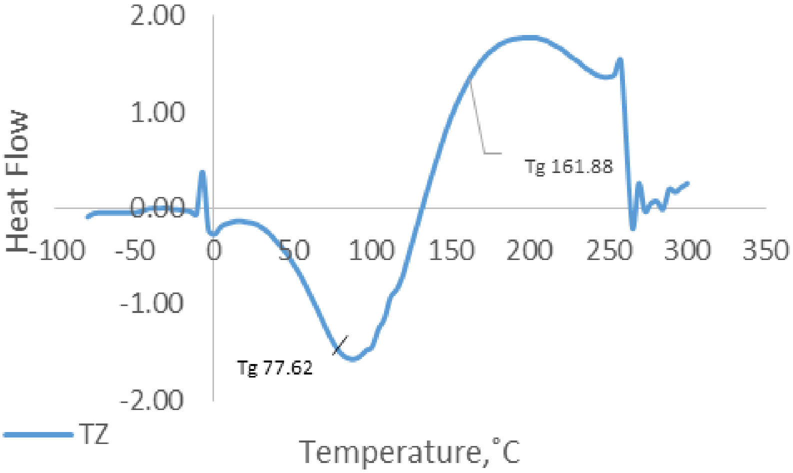


Figure 1: DSC Curve of Thermoplastic Zein

Figure 2 shows TGA curves for different multiphase systems include those of neat PCL and TZ. TGA curve of the TZ shows 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. In comparison, a steep weight decrease starts from 330 to 400 ˚C and can be related to the degradation of zein protein. However, the PCL curve shows the steep weight decrease begins from 400 to 450 ˚C due to the degradation of PCL.

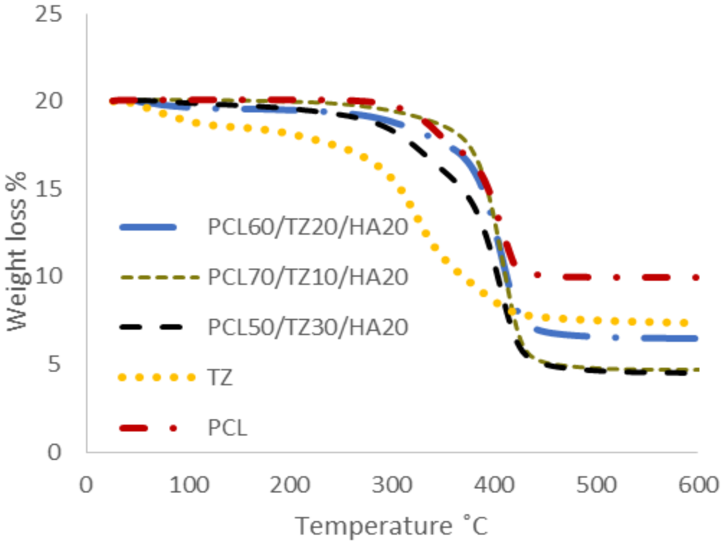


Figure 2: TGA Curves of Different Multi-Phase Systems Prepared Including Neat PCL and TZ for Comparison

Blended of three different materials with different compositions have a significant effect based on the TGA curves plotted. PCL_{50}-TZ_{30}-HA_{20} and PCL_{70}-TZ_{10}-HA_{20} show a less 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 is due to the different composition of TZ and PCL used in the system prepared instead of HA, which remains the same for the overall composition. These results proved the heterogeneous nature of the system prepared.

Figure 3 shows the DSC curve of the three different multi-phase systems to reveal the presence of thermal transition occurs in the systems. DSC curve for PCL-TZ-HA shows a T_g appeared when the temperature around 50˚C. The polymer which undergoes T_g causes the physical and chemical properties of the polymer to be changed significantly. The thermal analysis on the composition of PCL-TZ-HA provides useful information for the selection of processing temperatures in foaming (e.g., solubilisation and foaming temperatures). The gas foaming temperature was selected with the minimum temperature close to T_g. Salerno [17] observed the temperature below the T_g of the Thermoplastic Gelatin (TG) causes the foaming to be retarded due to the steep rigidity of the polymer. T_g is the phenomenon of the amorphous polymers and amorphous portion of semi-crystalline polymers. The endotherm curve appears on the DSC curve is mainly due to the melting of crystalline polymers.

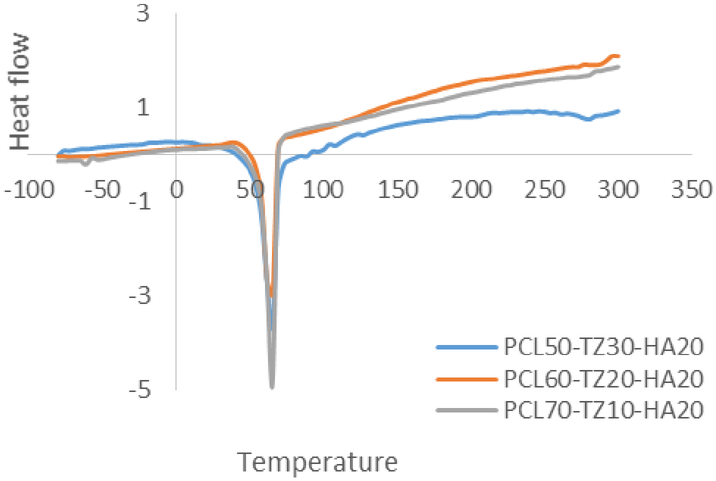
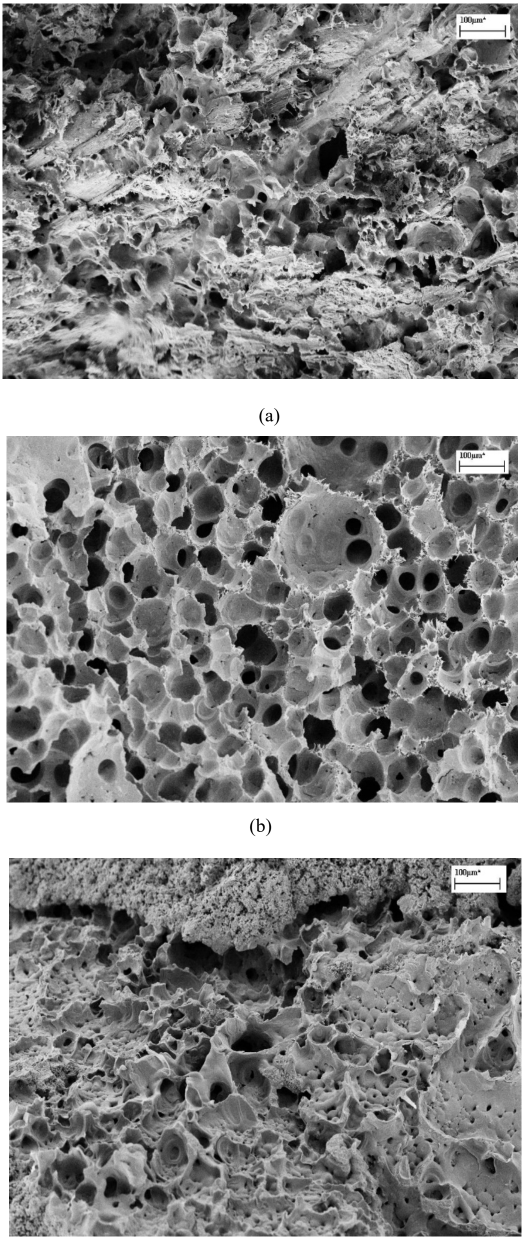


Figure 3: DSC Curve of Different Multi-Phase Systems Prepared for Comparison

Supercritical CO_2 foaming of PCL/TZ/HA Bio Composite

Supercritical CO_2 foaming tends to plasticise 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 [9]. Selection of proper operating conditions mainly blowing agent type (CO_2, N_2, and or their mixture), foaming temperature, saturation pressure, and pressure drop rate have a broad effect on the pore structure features of porous materials prepared via $scCO_2$ foaming process. Figure 4 shows the SEM morphology of the cross-section of PCL/TZ/HAp of different composition foams. The specimens have fixed their processing parameters such as saturation pressure equal to 20 MPa, 50 ˚C, foaming for six hours, and high depressurised. The selection of the foaming parameters process brings the target for perfect growth on the multiphase systems [9, 11].



(a)

(b)

(c)

Figure 4: SEM Micrograph Images of the Cross Section of (a) $PCL_{70}/TZ_{10}/HA_{20}$ (b) $PCL_{60}/TZ_{20}/HA_{20}$ (c) $PCL_{50}/TZ_{30}/HA_{20}$

From Figure 4, it clearly shows that the heterogeneous distribution on the size of the pores was promoted by the foaming process. The foaming temperature selected (50˚C) was justified since all the PCL/TZ/HA bio composites were thermally stable up to 250 ˚C, as shown in Figure 2. The supercritical CO_2 foaming of plastics requires that CO_2 diffuse into the material at supercritical conditions. According to the literature, the results obtained at a lower temperature of foaming cause the TZ foams, indicating small cells separated from each other by thick walls, generating highly irregular and rough pore surfaces. [17]. The low foaming temperature used in $scCO_2$ was significantly lower than the TZ degradation temperature (323.9 ˚C), which preserves the chemical structure of the thermoplasticised 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 pore size was observed after the increase of the depressurisation time from 15 to 120s [11]. The strong obvious interconnected of the porous structure was built from the composition of $PCL_{60}/TZ_{20}/HA_{20}$ (Figure 4 (b) compared to the (Figure 4 (a)) where the effect of foaming process on $PCL_{70}/TZ_{10}/HA_{20}$ shows low interconnection, ascribed to the homogeneously distributed HA particles throughout the mass of PCL matrix. The distribution of the HA particles within the mass of the scaffolds is an important concern. Similar results were obtained from the literature indicating a minor effect of the organic filler on the density and porosity of the foams PCL/HA [9]. Figure 4 (b) and Figure 4 (c) give clear SEM morphology of less porous distribution on the composition structure. This happens due to non-equilibrium diffusion of CO_2 on the surface of the material structure. The position of the material composition inside the vessel while conducting the $scCO_2$ plays a vital role in stabilising the diffusion of CO_2 within the surface materials.

CONCLUSION

The incorporation of thermoplastic zein at different concentrations has a significant effect on the thermal properties of PCL/TZ/HA bio composite. All the PCL/TZ/HA bio composites are thermally stable up to 250 ˚C. Moreover, 20 wt% of thermoplastic zein showed better pore structure morphology of porous PCL/TZ/HA bio composite compared to others.

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