Thermal analysis on Characterization of Polycaprolactone (PCL) – Chitosan Scaffold for Tissue Engineering

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ABSTRACT
Tissue engineering (TE) is a multidisciplinary field focused on the development and application of knowledge and information to the solution of critical medical problems, as tissue loss and organ failure. The defects of human tissue, organ failure, injuries or any types of damage are one of the most problems in human health care. This paper is a study on thermal characterization on polycaprolactone (PCL)-Chitosan (CHT) biocomposite used for tissue engineering application. The multi-step process started with preparation of bio-composite by using PCL, CHT and Dichloromethane (DCM) as a solvent via solvent casting technique. The PCL-CHT bio-composites were characterized using thermo-gravimetric analyser (TGA), differential scanning calorimetric (DSC) and x-ray diffractions (XRD). Thermal characterization were analysed by TGA analysis DSC analysis. The X-Ray Diffraction results showed sharp peaks and high intensity which confirms the PCL-CHT biocomposites crystallinity microstructure. The blending of biocomposite scaffold changed the crystallinity structure compared to single material. PCL-CHT biocomposite scaffolds exhibit a better thermal stability compared to pure PCL.

Keywords *- Chitosan, Polycaprolactone, Solvent Casting Technique, TGA, DSC, XRD*

I. INTRODUCTION

In general, scaffolds are biodegradable polymeric porous structures with pre-specified shape and are mainly used for tissue engineering to repair or replace the damaged tissue in the body and also to provide mechanical support. The parameters requirement and considerations are mechanical properties, porosity, drug release behaviour, cell growth, morphology and biocompatibility. However, thermal properties are necessary in order to aid the preparation processes. A high porosity and a high interconnectivity of the scaffold between the pores are necessary to allow cell growth and flow transport of nutrients and metabolic.[1] In the development of biomedical fields, biodegradable polymers are importantly promoted because of their biocompatibility and biodegradability. More research is ongoing in order to obtain a novel biodegradable polymer with specific properties.[2]

Biomaterials and fabrication technologies are a most important factor in TE. Designed materials must be suitable to excite specific cell at molecular level. Specific interactions with cell should be elicit and so direct cell attachment, extracellular matrix production, propagation and organization. Selection of materials is the most point to obtain successful of TE practice other than that is the requirements such as biocompatible and the mechanical properties of biomaterials to be use. [3, 4]

For biomedical scaffolds, fact stated that single biodegradable polymer definitely not encounter all the requirements whereas numerous polymeric materials are available and have been examined for TE. Because of that, fabrication of various component polymer systems characterize a plan to improve advanced, efficient and multi-useful biomaterials.[5] Particularly, nanostructures in biodegradable polymer matrices are to get nanocomposites with specific properties that capable used in TE. Nanoscale is defined for basic functional of tissues and cells sub-unit. Nanotechnology and nanobiology have to understand clearly since it represented a novel limit in TE research.[6]

Scaffolds should be biocompatible, resorbable and mechanically stable to supply temporary support to the implanted cells.[7] Normally, required scaffold made from polymers, ceramics or composites must own specific characteristics and properties including porous, high surface area, good structural strength, particular three dimensional shapes and biodegradability.

Several of polymeric materials either natural or synthetic polymer scaffolds are being verified for tissue

regeneration and repair.[8] Design factors are the most considerations in scaffold fabrication for TE. Several considerations such as mechanical strength, biocompatibility, cell affinity are the important while choosing materials in order to meet the TE applications and capability to support cartilage tissue formation and flexible biodegradability.[9] In polymerics scaffolds, the ideal characteristics such as rate of degradation, porosity, strength, microstructure, shapes and sizes are ready in developing scaffolds.[10]

Blending of synthetic and natural polymers can produce various processing techniques and physicochemical properties of synthetic polymers. For example, the blending of polyhydroxyethyl methacrylate with gelatin was mitigated the poor cell adhesion.[11] In TE, realization of scaffold with mechanical, physical and biological properties are one of the important issues. Scaffolds can support for tissue formation and act as substrate for cellular growth and propagation.[5]

Bioactive ceramics and biodegradable polymers are being combined in a variety of composite materials for tissue engineering scaffolds. Synthetic bioactive and bioresorbable composite materials are important as scaffolds for tissue engineering. The use of composite scaffolds can provide unique biological and biomechanical properties for the development of tissue engineering scaffolds functions. Sarasam et al. showed that the blending of chitosan with PCL gave a superior biomaterial and the limitation reacted by PCL. As previous study, the advantages of Chitosan include positive charge, cheap and easy to find created many attention due to its various benefits as well as biocompatibility and anti-microbial activity.

Poly (ε-caprolactone), PCL is a synthetic biodegradable polymer that has widely range of uses in TE.[12] Since PCL is expensive, blending of PCL with other cheaper copolymers may reduce the cost and can get the final product suitable and leading to commercialize.[13] PCL is extremely processes because it is dissolve in organic solvents and has a low melting point at 55°C – 60°C and glass transition temperature is at 60°C. Besides, PCL able to form miscible blends with wide range of polymers. Originally, PCL was examined as a long-term drug due to the properties of the PCL itself includes non-toxicity, slow degradation and high permeability for several drugs. General research is ongoing widely to develop long-term drug with micro-sized and nano-sized based on PCL. Because of the excellent biocompatibility of the PCL, extensive investigation been done as a scaffold for TE.[1]

The property of solvent casting preparation of scaffolds is very simple and low cost. Preparation can use simple equipment, not require major equipment and up to the evaporation of solvent to form the scaffolds by one of the two routes.[14] The other technique which is particulate leaching technique was developed to enhance control over pore diameter and porosity as compared to most fabrication methods.[15] From the previous study showed that fabricated chitosan-PCL copolymer scaffolds achieved a microstructure by a true gradient and relatively change the pore size and porosity continuously along the longitudinal direction through combination of both methods layer-by-layer assembly and a particulate-leaching method.[16]

Previous study stated that chitosan blending is naturally developing polysaccharide with a synthetic polymer.[11] In forming novel biomaterials, the flexible processing conditions PCL offers have not been fully exploited. A number of blending chitosan and PCL were studied such as in organics solvent, in acidic water or in mix solvent of water and acetic acid that resulted advantage of polymers blending. Therefore, PCL were used for long-term implants because of the suitable materials and properties.[17] A study about fabrication of porous PCL/chitosan blend scaffolds. [18] The design and fabrication of scaffolds are by particle leaching technique using hexafluoro-2-propanol as solvent and salt particles as porogen. This researcher also mentioned that this technique might not be suitable to fabricate scaffolds with small pore sizes and low porosities due to the fact. This study was used not higher than 50 weight percent of chitosan to maintain sufficient strength of the resultant scaffolds. So this study concentrates on characterization of the PCL-CHT scaffold prepared by using solvent casting technique.

2. EXPERIMENTAL

2.1 Materials

Polycaprolactone (PCL) (e-Sun™) off-white colour resin and Chitosan (KiOnutrime®-CsG) fine free flowing powder used as a biofiller (with the particle size approx.84µm) were purchased from Innovative Pultrusion Malaysia Sdn. Bhd., Malaysia. Dichloromethane (DCM) from Quassi-S Pte. Ltd. was used as a solvent. DCM molecular weight is 60.05g/mol.

2.2 Thermogravimetric analysis (TGA)

The TGA test set up consisted of a thermogravimetric analyzer Q500 TA instrument was used to analyze the thermal properties and the degradation of PCL/Chitosan biocomposites scaffolds. TGA testing was done at heating rate of 500°C/min from 38°C to 850°C. Biocomposites scaffolds samples will degrade as the

heat rise and weight of the samples also loss due to the degradation rate.

2.3 Differential scanning calorimetry (DSC)

DSC analyses were done by using Differential Scanning Calorimetry (DSC) named Q200 TA instruments for four different compositions of PCL-CS samples. For the first cycle, the heating phase in a temperature range was from -70° C to -98° C with heating rate of 20° C/min. Then the second cycle was the cooling phase with a cooling rate of 20° C/min for temperature range from 98°C to -70°C. The same procedure was repeated for three times to obtained a total number of six cycles which consist of three heating and three cooling phase. DSC curve obtained was used to analyze the glass transition temperature (T_g), enthalpy (H_m), and crystallization phase (T_c).

2.4 X-ray Diffraction (XRD) analysis

Samples of PCL-CHT biocomposites were analyzed by using X-Ray Diffractometer (Shimadzu, XRD-6000). The experimental were set at voltage 30 kV by applied current of 20mA. Then the operations were set for drive axis 2θ angle range from 10° to 70°, step size of 0.02 and at a scan speed of 3°/min. The reflected intensities were recorded at 2θ scattering angle.

3. RESULTS AND DISCUSSION

3.1 TGA analysis

Fig. 1 exhibits thermogravimetric (TG) of PCL, chitosan and PCL/chitosan scaffolds. The decomposition temperature for each step and the residue of scaffolds biocomposite PCL and PCL-Chitosan blend fibers are observed from Fig.1 The TGA graph is used to analyze the decomposition temperature and amount of residue (%) and also the weight change (%) respect to temperature (°C). From the graph, it showed that PCL scaffold completely discomposed which begins at 369°C in a single stage. Weight loss of two-stage chitosan scaffold was verified. At first stage, scaffolds showed the weight loss at 110°C which represented the moistness evaporation inside scaffold and for the second stage started at 287°C. From the observations, it also showed that thermal degradation region related to the complete process which is includes the dehydration of the saccharide rings. Then the process monitored by decomposition of chitosan. The curves show a shift to a higher temperature for PCL-Chitosan because of the Chitosan presence. It means that PCL-Chitosan exhibit a better thermal stability compared to pure PCL. The TGA curves also showed the three stage degradation behavior for the blend scaffolds PCL-Chitosan biocomposite. For the first stage, the degradation step occurs before 105°C, it should be the loss of water that bound in chitosan element and removal of any amount of acetic acid left inside the scaffolds. Typical thermal degradation characteristics of PCL and chitosan elements are reflect at second and third stages correspondingly. [12]

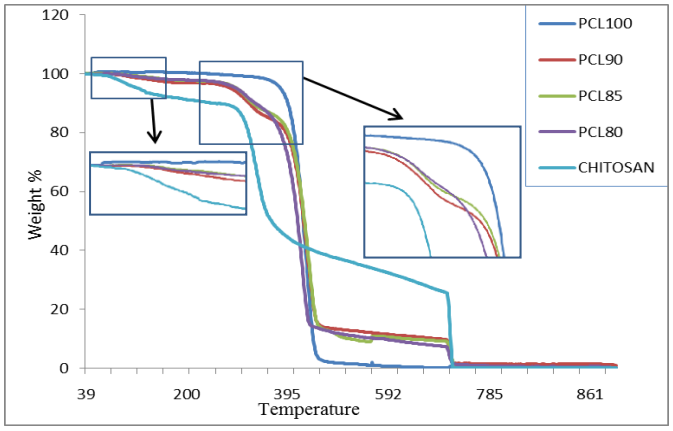

Fig. 1 TGA curves for PCL and PCL-Chitosan with various compositions

From the results, it shows that the thermal stability of biocomposite has increased. The increase in thermal stability attributes stability of PCL.

3.2 DSC analysis

Chitosan and PCL are crystalline polymers. Differential Scanning Calorimetric study showed the miscibility properties of the scaffolds. Blending crystalline polymer with other polymers will provide immiscibility due to the depression of melting point. Melting temperature for pure PCL is at 60 ºC and the glass transition temperature is at -60 ºC around. However, chitosan starts degrading at 257 ºC preceding to melt. Thus, PCL melting point was observed to examine the miscibility of the polymers blend. Fig. 2 showed PCL decrease in the melting temperature (T_m) but PCL-CHT increases when the compositions of chitosan increase. The decreasing of T_m happened might be due to the chemical interaction between PCL and CHT in composite.

In principle of DSC analysis, if blending of two components and totally miscible each other, then new T_g would be observed between the original T_g of elements in the DSC thermogram of the blend. Then, if partly miscible, the results for blending would have two T_g related to the each element, but measured T_g values equivalent to each element that could be affected each other which reliant on the composition ratios.

The DSC curve in Fig.2 and Fig. 3 were showed that the endothermic melting peak (T_m) for PCL biocomposite scaffold is 57.98 ℃ and involved a glass transition temperature of 55.18 ℃. This result indicates the melting temperature for PCL biocomposite scaffold is lower than pure PCL pellets. The T_g of PCL-CHT biocomposites were slightly affected while blending with chitosan. The PCL-CHT sample with 20% CHT content showed higher value than both PCL-CHT sample with 10% and PCL-CHT 15%. This may due to the interaction of polymer chains with the surface of particles that can change chain kinetics in the region immediately surrounding the particle due to presence of interface.

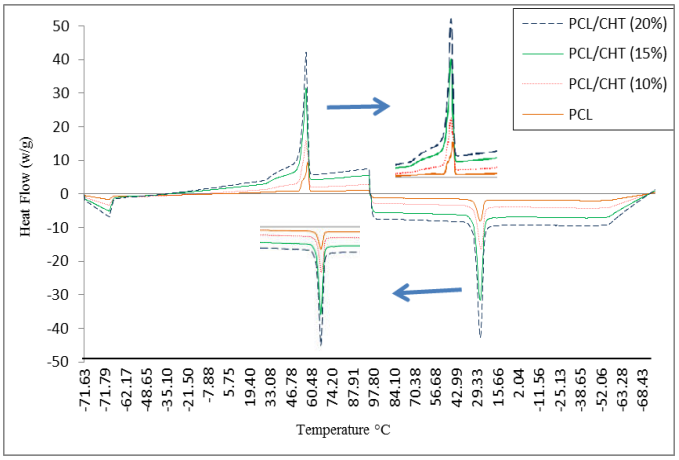

Fig. 2 DSC thermograms for PCL and PCL-CHT biocomposites with various CHT content during heating time.

Fig.3 showed that there is no any thermal result for the T_g of PCL although PCL membranes were scanned and starts from −71°C.

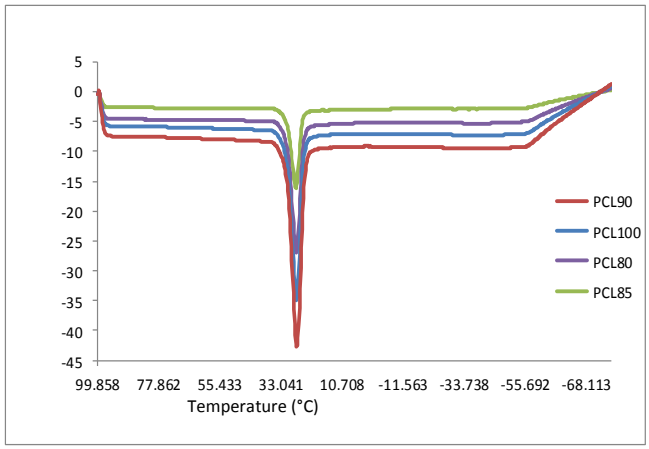

Fig. 3 DSC thermograms for PCL and PCL-CHT biocomposites with various CHT content during cooling time.

The data for T_m, T_g, T_c for PCL biocomposite scaffold and PCL-CHT scaffold with various composition of CHT are observed from the Fig.3. It was observed that the T_m of PCL element decreases in proportional to the increase in composition ratio of chitosan.

The DSC thermogram of PCL-CHT with various composition of CHT content almost repeat same thermal behaviors of PCL components and can be used to locate a new T_g.

3.3 XRD analysis

The XRD measurement confirms the ion induced loss of crystallinity in PCL samples. The crystalline state of scaffold PCL-CHT biocomposite as a function of chitosan concentration was examined by XRD analysis. The X-ray diffraction of pure PCL, PCL-Chitosan (10%), PCL-Chitosan (15%) and PCL-Chitosan (20%) are shown in Fig. 4 respectively.

For pure PCL, a number of crystalline peaks were also seen. The X-ray diffraction patterns shows two well resolved diffraction peaks. For main peak, (110) plane was referred and weak plane is at (200) plane. The main diffraction occurs at 2θ=21.9º and the weak peak occurs at 2θ=24.2º.[13] This can be attributed to the semicrystalline of PCL polymer. Meanwhile, the two blends for PCL-Chitosan (10%) and PCL/Chitosan (20%) are produced extra peak at about 24.3º as shown in Fig. 4. Besides, the intensity of extra peak at 24.3º in the PCL-Chitosan (10%) spectrum showed higher compared to pure PCL. Results for blending samples occur may be cause by changing of the coordinate property of the chitosan molecules.

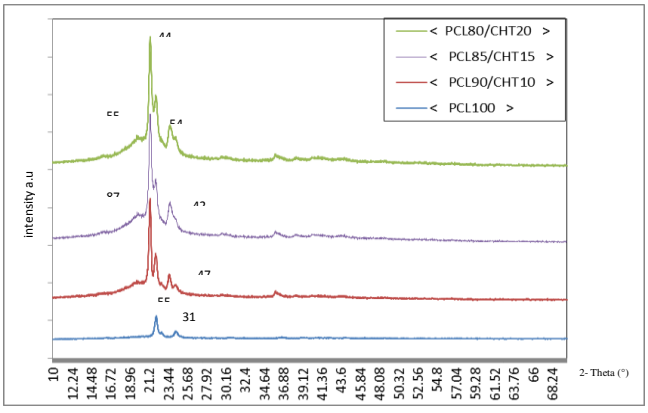

Fig. 4 Pattern of XRD of PCL-CHT composite with different wt%. of chitosan loading (0, 10, 15, and 20).

4. CONCLUSIONS

The biocomposite were prepared from PCL and CHT by using Solvent casting technique. The analysis on the X-Ray Diffraction showed sharp peaks and high intensity which confirms the PCL-CHT biocomposites scaffolds crystallinity microstructure. The PCL-CHT biocomposites scaffolds amorphous phase was demonstrated by the low and wide intensity peak. The blending of biocomposite scaffold changed the crystallinity structure compared to single material and can be concluded that both amorphous and crystallinity existed in PCL-Chitosan. Thermal characterizations were analysed TGA and DSC analysis. PCL-Chitosan biocomposite scaffolds exhibit a better thermal stability compared to pure PCL.

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