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PREPARATION AND CHARACTERIZATION OF PHOTO-CURABLE PCL/PEG-DIACRYLATE FOR ADDITIVE MANUFACTURING TISSUE ENGINEERING SCAFFOLD APPLICATION

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ABSTRACT: PCL is one of important tissue engineering scaffold materials. Besides traditional scaffold fabrication methods, additive manufacturing (AM) techniques have also been used to provide controlled pore size and required geometry. Most AM approaches utilize extrusion or sintering to generate PCL scaffolds, yet few use photo-curing. In this research, photo-curable PCL (PCL-DA) was considered as the scaffold material, which will be photo-polymerized by visible light using a self-developed dynamic masking AM system. In order to improve its strength and hydrophilic character, PEG-diacrylate (PEG-DA) is added in the material system. Three different ratios (6:4, 7:3, 8:2) of PCL-DA to PEG-DA were prepared and characterized by differential scanning calorimetry (DSC), thermomechanical analyzer (TMA), tensile tests, water contact angles, and cell culturing. The results showed that the ratio of 6:4 is the best among the three. A preliminary study of scaffold fabrication was conducted by the AM system to demonstrate the feasibility of adopting photo-curable PCL/PEG-DA for tissue engineering scaffold application.

INTRODUCTION
Polycaprolactone (PCL) has been used as tissue engineering scaffold material for its biocompatible, non-toxic, and biodegradable characteristics. Some tradition scaffold fabrication methods were adopted to fabricate PCL scaffolds, such as particulate leaching by Reignier and Hunault (2006) and immersion-precipitation by Chung et al. (2011). Additive Manufacturing (AM) processes have become popular approaches to fabricate scaffolds with the advantages of unrestricted geometry and controlled pore size and distributions. PCL-based scaffolds built by AM technologies are mostly by extrusion and sintering. Works by Zein et al. (2002), Mondrinos et al. (2006), Seyednejad et al. (2011), Domingos et al. (2013) belong to extrusion type, while Williams et al. (2005) and Lohfeld et al. (2012) are examples using sintering approach. Literatures about photo-polymerized PCL scaffolds by AM technology are limited since photo-curable PCL is not commercially available and material synthesis is required. Kweon et al. (2003) and Elomaa et al. (2011) proposed methods to synthesize crosslinkable PCL macromer that can be UV polymerized to form PCL networks. In our previous research, Cheng et al. (2013), similar synthesis approach was adopted, and the photo-curable PCL (PCL-DA) was cured by visible light to form tissue engineering scaffolds by a self-developed Dynamic Masking AM System. However, the tensile...
strength is limited and PCL is hydrophobic that would affect cells attachment and proliferation. Hence, in this research, hydrophilic PEG-diacylate (PEG-DA) was added in the material system to improve the above issues. The formulations of three ratios of PCL-DA/PEG-DA were prepared and characterized in order to be used in additive manufacturing scaffold application. The thermal properties of the cured scaffold material were characterized by the differential scanning calorimetry (DSC) and thermomechanical analyzer (TMA), while the improvement due to PEG-DA was investigated through tensile tests and water contact angles. Moreover, cell culturing was conducted to compare the biocompatibility of cured material under different ratios.

MATERIALS AND METHODS

Synthesis of polymerizable PCL (PCL-diacylate)

In order to obtain polymerizable PCL (PCL-diacylate), PCL diol (average Mn=2000 g/mol) was reacted with dehydrated acryloyl chloride with the existence of triethylamine (TEA) serving as catalyst. The scheme of the synthesis is shown in Figure 1. The PCL diol would be encapped with acrylate groups at the ends to form PCL-diacylate (PCL-DA). The reaction was performed at 80°C for 3 hours, and then the triethylamine hydrochloride was removed by filtration. The precipitated PCL-DA was dried in vacuum at 40°C for 24 hours to attain final product.

Preparation of photo-curable PCL-diacylate /PEG-diacylate

In this research, PEG-diacylate (PEG-DA), serving as the crosslinking agent, was considered to mix with PCL-DA and photo-initiator to improve strength and hydrophilic character of the cured material. PEG-DA is hydrophilic, biocompatible, soluble in water and organic solvents, and photo-crosslinkable. Its C=C at both ends is expected to form stronger network with PCL-DA during photo-crosslinking, providing better mechanical properties for scaffold applications. Moreover, with the existence of hydrophilic PEG-DA in the material system, the hydrophobic effect from PCL is anticipated to be reduced. PEG-DA (Sigma-Aldrich Co. LLC.) with average molecular weight of 700 g/mol was selected in this study. Three ratios of PCL-DA/PEG-DA (6:4, 7:3, 8:2) were prepared and investigated in this research. Since the material will be cured by visible light from a DLP projector, TPO (Chembridge International Corp., Taiwan) was selected to be the photo-initiator to match the target spectrum range. Acetone was used as solvent to mix PCL-DA, PEG-DA, and TPO. The detailed formulations are listed in Table 1.

![Figure 1 The scheme of synthesizing polymerizable PCL-diacylate (Cheng et al., 2013)](image)

Table 1 Three formulations of photo-curable PCL-DA /PEG-DA material system

<table>
<thead>
<tr>
<th>PCL-DA : PEG-DA</th>
<th>PCL-DA (g)</th>
<th>PEG-DA (g)</th>
<th>Photoinitiator, TPO (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 6:4</td>
<td>0.6 g</td>
<td>0.4 g</td>
<td>0.02 g</td>
</tr>
<tr>
<td>2 7:3</td>
<td>0.7 g</td>
<td>0.3 g</td>
<td>0.015 g</td>
</tr>
<tr>
<td>3 8:2</td>
<td>0.8 g</td>
<td>0.2 g</td>
<td>0.01 g</td>
</tr>
</tbody>
</table>

Acetone: 0.7 ml
Photoinitiator is 5wt% relative to PEG-DA.
**Differential Scanning Calorimetry (DSC)**

DSC (DSC 4000, PerkinElmer Inc., U.S.A.) was utilized to evaluate the thermal properties of three formulations with different ratio of PCL-DA/PEG-DA. DSC can measure temperatures and heat flows associated with thermal transitions in a material. The measurement was carried out at a scanning rate of 20°C/min and nitrogen gas flow rate of 20 ml/min. In particular, melting point was determined at the maximum of the melting endotherm.

**Thermal Mechanical Analysis (TMA)**

In order to understand thermal expansion properties of the cured scaffold material at regular human body temperature, a thermomechanical analyzer (TMA 2840, TA Instruments, U.S.A.) was adopted to characterize the linear expansion among 35~37°C. The samples were heated at the rate of 1°C/min and the slope of the measured data at the specified temperature is the thermal expansion coefficient of the material at that temperature.

**Tensile test**

The tensile test adopted the ASTM D638-10 Type V standard, and three samples were tested for each formulation. For comparison, PCL-DA without adding PEG-DA was also tested. The specimens were built through molding approach instead of Additive Manufacturing system due to the larger dimensions than the system’s build envelope. An external light source (TFO TL-808, Taiwan Fiber Optics, Inc., Taiwan) which is similar to the light from DLP was used to cured the specimens. The tensile tests were performed by INSTRON 3365 Tabletop Universal Testing System (Instron, U.S.A.). The speed of testing was set to be 1 mm/min.

**Water contact angle**

Contact angle was measured to evaluate the hydrophilic character of the materials. For a hydrophilic material, contact angle is less than 90 degrees, and a smaller angle indicates better hydrophilicity and better wettability. Tangent method, in which the contact angle (θ) is the angle formed by the solid surface and the tangent of the droplet (Figure 2), was adopted for measurement. Since the shape of the droplet was assumed to be part of outline of a circle, a half-angle (θ/2) method can be utilized. That is, the angle (θ₁ or θ₂, Figure 3) of straight line connecting the left/right end and the apex of the droplet against a solid surface is θ/2. Contact angles calculated from left and right ends were averaged to obtain the final result. The measurement setup is shown in Figure 4, where the water droplet of 0.5ml was dropped on the sample staying for 5 seconds and a CCD camera caught images for further calculation. Due to the small quantity of droplet, the effect of gravitational force can be ignored. Three samples were tested for each case. In addition to the three formulations, scaffold material without adding PEG-DA was also tested for comparison.

**Cell Culturing and MTT Assay**

For understanding the differences of biocompatibility for various scaffold material formulations, cell culturing with MTT viability test was performed. L929 fibroblasts cells were seeded onto membrane of scaffold materials in 24-well plates with 5×10⁴ cells/well initially, and cultured for 1,
Cells cultured in the wells without scaffold materials served as the control group. MTT (3-[4,5-cimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay was employed to evaluate cells viability. The optical density (O.D.) values were read at 570 nm. Three samples of each formulation were measured and averaged for comparison.

RESULTS AND DISCUSSIONS

Differential Scanning Calorimetry (DSC)
The DSC results of cured PCL-DA/PEG-DA scaffold materials were summarized in Figure 5. The melting point can be determined from the plot. For comparison, samples of PCL diol and synthesized PCL-DA were also tested (Figure 6). The melting point of PCL-DA (56.07°C) is lower than that of PCL (58.09°C) due to the existence of acrylate groups that decrease the crystallinity. Similarly, adding PEG-DA in the material system also resulted in lower melting points in three formulations compared to PCL-DA only. The ratio of 6:4 has the highest melting point (54.84°C) among the three. The melting points are all above normal human body temperature range, which indicates that the materials are thermally stable for human body environment applications.

Thermal Mechanical Analysis (TMA)
Based on the TMA results, the coefficients of thermal expansion (CTE) at 37°C can be calculated (Table 2). The scaffold material of 6:4 ratio has the smallest CTE at 37°C and will have least dimension changes in human body environment due to temperature variation.

Tensile test
The tensile test results are listed in Table 3. Adding PEG-DA in the material system serving as the crosslinking agent did help to form stronger network after photo-curing and was proved to improve mechanical properties effectively. The more PEG-DA were added, the higher tensile strength and Young’s modulus were achieved. The cured material with 6:4 ratio performed the best among the three, which is 6.2 times stronger than the cured PCL-DA without PEG-DA.

Water contact angle
The results of average contact angles for different formulations are compared in Table 4. The existence of PEG-DA in the material system did enhance the hydrophilicity of the scaffold materials as expected. A smaller contact angle, meaning better hydrophilic character, was obtained when more PEG-DA were added.

<table>
<thead>
<tr>
<th>PCL-DA:PEG-DA</th>
<th>6:4</th>
<th>7:3</th>
<th>8:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of thermal expansion (µm/°C)</td>
<td>288</td>
<td>265</td>
<td>236</td>
</tr>
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</table>
Table 3 Average Strength and Young’s Modulus from tensile test.

<table>
<thead>
<tr>
<th>PCL-DA/PEG-DA ratio</th>
<th>Strength (MPa)</th>
<th>Young’s Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6:4</td>
<td>0.81</td>
<td>16.59</td>
</tr>
<tr>
<td>7:3</td>
<td>0.59</td>
<td>10.49</td>
</tr>
<tr>
<td>8:2</td>
<td>0.45</td>
<td>7.43</td>
</tr>
<tr>
<td>PCL/DA without PEG-DA</td>
<td>0.13</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Table 4 Average Contact angle results for different formulations.

<table>
<thead>
<tr>
<th>PCL-DA:PEG-DA</th>
<th>$\theta_1$</th>
<th>$\theta_2$</th>
<th>Contact angle $\theta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6:4</td>
<td>19.57°</td>
<td>18.49°</td>
<td>38.06°</td>
</tr>
<tr>
<td>7:3</td>
<td>22.66°</td>
<td>22.93°</td>
<td>45.59°</td>
</tr>
<tr>
<td>8:2</td>
<td>27.35°</td>
<td>28.40°</td>
<td>55.75°</td>
</tr>
<tr>
<td>PCL-DA without PEG-DA</td>
<td>38.99°</td>
<td>41.02°</td>
<td>80.01°</td>
</tr>
</tbody>
</table>

Cell Culturing and MTT Assay

The MTT assay results are shown in Figure 7. The data which are obviously outliers were removed during calculation. After 1 day, cells showed great proliferation in the samples of 6:4 ratio and comparable viability to the control group in the samples of 7:3 and 8:2 ratios. After 3 days, cells growth in 6:4 samples still performed better than the control group. In contrast, the growth was limited in 7:3 samples and was declined in 8:2 samples. After 5 days, the O.D. values of three formulations dropped rapidly to similar range, which is possibly because the cells were over confluent in the plates. The scaffold material with more hydrophilic PEG-DA in the formula is favorable to cells viability and has better biocompatibility.

Preliminary Scaffold Fabrication

Based on the above characterization results, the ratio of 6:4 is the best among the three formulations. Therefore, we adopted this ratio to conduct preliminary scaffold fabrication. A self-developed Reflective Dynamic Masking Additive Manufacturing System, using a DLP projector as visible light source, was utilized. The scheme of the system is illustrated in Figure 8. The resolution of the system is 20 µm/pixel. Three various patterns with line width of 100 µm were stacking alternately for form a porous scaffold. The microscope result of a fabricated scaffold staking with three pattern layers is shown in Figure 9.
CONCLUSIONS
In this research, hydrophilic PEG-DA was proposed to integrate with a photopolymerizable PCL (PCL-DA) material system to improve strength and hydrophilicity for tissue engineering scaffold applications. Three ratios of PCL-DA/PEG-DA were prepared and investigated. Methods of DSC, TMA, tensile test, contact angle, and MTT assay were adopted to characterize material properties. The results verified the assumed benefits of adding PEG-DA in the PCL-based scaffold materials. Besides, properties of different formulations are compared and found that the ratio of 6:4 is the best among the three, indicating more PEG-DA is better. Moreover, a preliminary study of scaffold fabrication was conducted using the best material ratio of 6:4 by a self-developed Dynamic Masking AM System to demonstrate the feasibility of this application.

REFERENCES