SYNTHESIS AND CHARACTERIZATION OF PLLA-PEG-PLLA TRIBLOCK COPOLYMERS AS BIODEGRADABLE THERMOPLASTIC ELASTOMERS FOR PERIPHERAL NERVE REPAIR

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ABSTRACT

The peripheral nervous system (PNS) is a complex and comprehensive system consisting of numerous nerves outside the human brain and spinal cord [1]. The major function of PNS is to connect organs and limbs with the central nervous system (CNS) [1]. Each year, 200,000 patients in the USA are treated surgically for PNS injuries caused by stretch and compression injuries, trauma and other surgical procedures [2]. The transplantation of autologous nerve grafts is the gold standard for connecting nerve gaps that are a maximum of 5 mm in length [3]. However, the availability of donor nerves with appropriate length is extremely limited, the harvested donor nerves may mismatch the size of injured nerve, and an additional surgical site with associated risks is required.

Many studies reported that the experimentally and clinically confirmed biodegradable material graft modes are better alternatives for improving the defects. Some studies focused on collagen gels as nerve conduits for nerve regeneration, however the repair was limited to gaps less than 30 mm [4]. The goal in this research is to create a new thermoplastic elastomer biomaterial with controlled and appropriate biodegradation rate and time for NGCs in PNS repair. The use of biodegradable block copolymers consisting of both hard
hydrophobic and soft hydrophilic segment can provide a flexible, partially-hydrated and biocompatible biomaterial for on demand and on-site fabrication of cellular constructs for PNS repair at hospitals rather than in factories.

In this study, poly(ethylene glycol) (PEG; B blocks) and various degrees of polymerization of poly(L-lactic acid) (PLLA; A blocks) were synthesized via ring-opening polymerization to form ABA triblock copolymers. The chemical, thermal and mechanical properties were characterized by nuclear magnetic resonance (1H NMR), gel permeation chromatography (GPC), differential scanning calorimetry (DSC), and tensile failure testing. Average water swelling ratio and degradation time were also investigated.

Characterization results showed that longer PLLA block chains resulted in copolymers with a slower rate of degradation, a higher strength modulus, and a higher elongation at failure. The melting point and crystallinity of the copolymer also decreased. Degradable thermoplastic elastomers with appropriate melting point, water absorbability, and young’s modulus hold great promise for further application as NGCs for PNS repair.
# TABLE OF CONTENT

LIST OF FIGURES........................................................................................................ VII
LIST OF TABLES........................................................................................................... IX
NOMENCLATURE.......................................................................................................... X

1.0 INTRODUCTION..................................................................................................... 1

2.0 LITERATURE REVIEW.......................................................................................... 4
  2.1 Nerve guidance channel...................................................................................... 4
  2.2 Physical properties of nerve tube........................................................................ 5
  2.3 Thermoplastic elastomer.................................................................................... 6
  2.4 PLLA-PEG-PLLA block copolymers................................................................. 6

3.0 EXPERIMENTAL.................................................................................................... 9
  3.1 Material.............................................................................................................. 9
  3.2 Synthesis............................................................................................................ 9
  3.3 Characterization............................................................................................... 10

4.0 Results and Discussion.......................................................................................... 13
  4.1 $^1$H NMR............................................................................................................ 13
4.2 GPC.................................................................16
4.3 DSC....................................................................18
4.4 Equilibrium swelling study..............................................24
4.5 In vitro degradation study.................................................27
4.6 Mechanical properties......................................................31
5.0 CONCLUSIONS.........................................................38
6.0 RECOMMENDATIONS..................................................39
7.0 REFERENCES.............................................................41
LIST OF FIGURES

Figure 1.  (A) Repair of nerve lesion with autologous sural nerve; (B) Nerve tube repair.

Figure 2.  Different phase of nerve generation across nerve tubes.

Figure 3.  Schematic of different type of block copolymers.

Figure 4.  Water uptake of multiblock copolymers and homo-PLLA.

Figure 5.  (A) Structure of PLLA-PEG-PLLA copolymers; (B) $^1H$-NMR spectrum of 20K; (C) $^1H$-NMR spectrum of 20K-10.64.

Figure 6.  DSC thermograph of each type of synthetic copolymers.

Figure 7.  Onset melting temperature of different copolymers with different PLLA loadings. (A) 20K; (B) 20K-42.67; (C) 20K-19.54; (D) 20K-10.64; (E) 20K-4.17.

Figure 8.  Peak melting temperature of different copolymers with different PLLA loadings. (A) 20K; (B) 20K-42.67; (C) 20K-19.54; (D) 20K-10.64; (E) 20K-4.17.

Figure 9.  Melting enthalpy of 20K with various PLLA loadings. (A) 20K; (B) 20K-
42.67; (C) 20K-19.54; (D) 20K-10.64; (E) 20K-4.17.

Figure 10. Prepared copolymers samples after 24 hours swelled at 37°C under slow spinning rate. (A) 8K-36.6; (B) 8K-18; (C) 8K-11.7; (D) 20K-42.67; (E) 20K-19.54; (F) 20K-10.64.

Figure 11. The swelling ratio of samples. From left to right are 8K-11.7; 20K-19.54; 20K-10.64.

Figure 12. Average weight sweling ratio of each type of synthesized copolymers at each day.

Figure 13. Average mass change of synthesized copolymers at each day.

Figure 14. Stress strain curve of each type of representative copolymer.

Figure 15. Ultimate strain for each type of copolymer.

Figure 16. Ultimate stress for each type of copolymer.

Figure 17. Young’s modulus of each type of copolymer.

Figure 18. Comparison of PLLA block length and Young’s modulus for each type of copolymer.
LIST OF TABLES

Table 1: $^1$H-NMR results of synthetic copolymers.

Table 2: GPC result of the synthesized copolymers.

Table 3: Detailed DSC results of copolymers.
NOMENCLATURE

\[ T_{m,\text{onset}} \quad \text{Onset melting temperature, °C} \]

\[ T_{m,\text{peak}} \quad \text{Peak melting temperature, °C} \]

\[ M_n \quad \text{Number average molecular weight, g/mol} \]

\[ M_w \quad \text{Weight average molecular weight, g/mol} \]

\[ \Delta H_m \quad \text{Melting enthalpy, J/g} \]

\[ M_r \quad \text{Molecular weight of polymer repeat units, g/mol} \]

\[ \sigma \quad \text{Stress, MPa} \]

\[ \varepsilon \quad \text{Engineering strain} \]

\[ W \quad \text{Mass, g} \]
1.0 INTRODUCTION

Peripheral nerve system lesion is commonly caused by trauma, various side effects of different types of surgery [1]. Multiple studies had shown that short distance defects in human can be successfully treated by implanted artificial nerve tubes [3]. 3D printing has been widely used to overcome the poor ability of conventional techniques of controlling the scaffold architecture [5] etc. With 3D printing techniques, the size and shape of 3D printed nerve guidance conduits for patients injured nerve can be precisely controlled.

PLLA is a hydrophobic polymer. PLLA has excellent obviously advantages and disadvantages. The long degradation period and high hydrophobicity will limit it use in biomaterials. Poly l-lactic acid (PLLA) can be produced from renewable resources like corn and starch. It is also a safe material vastly used in packaging materials, like tea bags [6]. The mechanical properties of PLLA is highly depend on the molecular weight. Higher molecular weight PLLA can possess higher strain but less tensile strength. The degradation period of PLLA is ranged from six months to two years. And PLLA has poor biocompatibility with soft tissue [7]. To tailor the degradation period as well as mechanical stiffness for as an appropriate consideration of biodegradable materials for nerve guidance tube, another type of hydrophilic with good mechanical properties is needed.
Many studies focused on polyethylene glycol, PEG, a biocompatible hydrophilic polymer that can be introduced onto PLLA chains to create a copolymer with tunable biodegradability, thermal properties and mechanical properties by its composites to achieve being an ideal polymer for various applications, such as drug delivery or human body implants [8] & [9]. PEG can be soluble in water and it is a non-toxic material approved by Food and Drug Administration for the use in the human body [10]. Moreover, PEG can be excreted from the human body via kidney [11]. By introducing hard PLLA segment onto soft PEG segments, PLLA-PEG-PLLA, a triblock copolymer with adjustable biocompatibility and mechanical properties can be manipulated.

The plastic deformation can be prevented as triblock and multiblock copolymers consists of hard and soft segments and thermoplastic elastomer (TPEs) can be formed [12]. PLLA-PEG-PLLA particularly has soft PEG segments in the middle and hard PLLA segment at both ends. The PEG domains stay soft, provide the copolymer with elasticity where PLLA crosslinked hard domains, which allows the materials to be elastic but flexible. Additionally, ABA type block copolymers with hydrophilic B segments ensure the material to swelling some water, hydrophobic A segments ensures the material has a relatively longer degradation time as a hydrogel. The hydrogel system can absorb lots of water and maintain its 3D structure at the meantime. PEG as a soluble element of PLLA-PEG-PLLA
can enhance the water absorption by 50% mole of 40 mole% of PEG [13]. With controlled PEG and PLLA element in PLLA-PEG-PLLA block copolymers system, a controlled degraded, partially hydrated materials with good mechanical properties and biocompatibility can be fabricated than PLLA homopolymer.

Many researchers have found many methods to synthesize PLLA in the presence of PEG with selected catalysts. The copolymers are synthesized by ring-opening polymerization (ROP) and initiated by the two hydroxyls end groups of PEG. Regular PEG has two hydroxyl end group which ensure to product a triblock copolymer after ROP. Many catalysts are being widely used in the PEG, PLLA copolymerization, stannous octoate (Sn(Oct)₂) is a commonly used one for PLLA added onto both PEG chains ends in ROP. ROP usually produce water as a by-product of the reaction, the reaction temperature usually carried out above the boiling point of water for the reaction to proceed further. Wan et. al., ca prepared PEG PLLA multiblock copolymers at 140 hrs for 24 hrs. Zhang et. al., synthesized PLLA-PEG-PLLA copolymers by using microwave-assisted synthesize [14].
2.0 LITERATURE REVIEW

2.1 Nerve guidance channel

The gold standard for peripheral nerve defects repair still uses the autologous nerve graft from sural nerve (Figure 1.A) [5]. The disadvantage of this method is obvious that extra incision is required and the size of donated nerve and injured nerve may not perfectly match with each other. A nerve tube (Figure 1; B) with right-off-the-shelf advantage ensure that nerve tubes with different sizes can be better used in nerve injuries repairs.

Figure 1. (A) Repair of nerve lesion with autologous sural nerve. (B) Nerve tube repair. [5]
2.2 Physical properties of nerve tube

Permeability is another critical element for axons or other types of cells to transport through the tube for reaching the site of regeneration [5]. The size of pores could also affect physical properties of nerve tube material. Other physical properties such as degradation and swelling may affect the nerve regeneration in a negative way by continuously releasing acid product (lactic acid) and poor tensile properties. With appropriate physical properties,
the nerve tube should also be flexible to maintain its 3D structure and strong enough to resist the external force.

2.3 Thermoplastic elastomer

The interests of biodegradable polymers grow in clinical use. In addition to the indispensable attribute of its biodegradable behavior. The extendibility of biodegradable polymers limits its application. Highly flexible biodegradable elastomers is developed. Using thermoplastic elastomers (TPEs) copolymers can enhance the extensibility of the biodegradable materials. TPEs are materials which are capable of elastic state at low temperature and melts at the higher temperature just like traditional thermoplastic materials. The ability of recycling and remodeling provides the opportunity to be fabricated on demand and on-site scaffolds for specific patients in hospital. The flexible segments ensure elasticity and hard segments prevents the copolymer from deformation. Many PLA-B-PLA (B=soft segment) have been studied including polyesters, polyethers and polycarbonate [7]. The biodegradable polymers with hard A segment and soft B segment could be capable as formed flexible and biodegradable properties for nerve guidance channels.

2.4 PLLA-PEG-PLLA block copolymers

As an ABA type triblock copolymers, PLLA-PEG-PLLA triblock copolymers with hard A (PLLA) segments and soft B (PEG) segment are categorized due to the structure. The
copolymers with different structures are shown in Figure 3.

![Schematic of different type of block copolymers](image)

**Figure 3. Schematic of different type of block copolymers [15].**

Luo et al. synthesized PLA-PEG multiblock copolymers with determined block lengths via bulk polymerization to study controlled drug delivery [16]. However, the melting point of those copolymers were lower or closer to 37 °C, which is not ideal for as an implant into body.

Chen et al. [17] synthesized both multiblock and triblock copolymers with PEG has
$M_n=2000$ and $4000 \, g/mol$ and studies its hydrophilicity as shown in Figure 4.

![Figure 4. Water uptake of multiblock copolymers and homo-PLLA[17].](image)

The water uptake is one of the considerations that affects the properties of the copolymer. As shown in the above figure, with the lower molar ratio of PLLA/PEG, more water the copolymer can absorb than higher PLLA/PEG molar ratio [17].

PLLA-PEG-PLLA has a potential application as the implant material due to its mechanical properties, hydrophilicity and biodegradability. As an extrudable thermoplastic elastomer, its hydrophilicity will affect the biocompatibility since the continuous released lactic acid and provide tunable biodegradability.
3.0 EXPERIMENTAL

3.1 Materials

Pharmaceutical-grade poly(ethylene glycol) (PEG) ($M_n = 20,000$ g/mol) were purchased from Polysciences, Inc. 3,6-Dimethyl-1,4-dioxane-2,5-dione (L-lactide), stannous octoate (Tin(II) 2-ethylhexanoate) and HPLC-grade toluene were purchased from Sigma-Aldrich. PEG and stannous octoate were stored at room temperature. HPLC-grade toluene was stored at room temperature in a flammable cabinet. L-lactide was stored in the refrigerator with the setting temperature between 2 to 8 °C to prevent self-polymerization. HPLC-grade toluene was dried by using molecular sieves several days prior to experiments. HPLC-grade water and methanol were purchased from Sigma-Aldrich and mixed as GPC mobile phase. Mobile phase was mixed with volume fraction of 20% water and 80% methanol.

3.2 Synthesis

12.5 mL pre-dried toluene was pipetted with a glass pipette into a 500 mL three-necked round bottom flask to dissolve PEG. 1.250 g PEG was measured by electronic scale and added into the flask. The system was purged with vacuum and nitrogen gas overnight to ensure all the volatiles were totally removed. 8.9 mL pre-dried toluene was pipetted into the system followed by 0.890 g L-lactide. The toluene was added into the flask on the base
of 10 mL per gram of monomer. Stannous octoate was pipetted into the flask at the ratio of 20% of the total mass of monomer. The concentration of L-lactide was controlled to obtain different block lengths and loading of PLLA onto both of the PEG ends. For relatively shorter and longer PLLA loading copolymer, 0.450 g, 1.780 g and 3.560 g of L-lactide was added, respectively. The system was purged with vacuum first for one minute and then purged with nitrogen gas. This procedure was repeated three times to make sure that the oxygen and moisture were removed from the system. Then the mixture was heated up to 140 °C in an oil bath and refluxed for 14 hours. A Schelenk line was used to regulate the vacuum and nitrogen gas line connected to the system. After the synthesis, the system was cooled down to room temperature for several hours and then the product was dried by using a rotary evaporator to remove all of the toluene. The dried product was dissolved in dichloromethane (CH₂Cl₂) and precipitated into 10x excess cold diethyl ether. The filtrated copolymers were collected in scintillation vials and dried at room temperature in a vacuum oven at least 24 hours. All dried copolymers were ground into powders prior to analysis and further study.

3.3 Characterization

TOSOH EcoSEC HLC-8320 GPC was used to determine the detailed molecular weight of copolymers. The number average molecular weight (Mₙ), weight average
molecular weight ($M_w$) and polydispersity indexed (PDI) were measured three times for each copolymer, respectively. The solubility of copolymers in the GPC mobile phase at the GPC operation temperature (40 °C) was tested prior to analysis. The calibration curve was carried out using PEG standards with molecular weights from 895-62300 g/mol.

A TA Q2000 was used to obtain DSC results of each copolymer. All copolymer samples were run in triplicate and prepared in sealed DSC aluminum pans with lids. The range of the sample masses were from 18mg to 21mg. The analyzing procedure was set up as a heat-cool-heat procedure. This procedure was carried out with a heating rate of 5 °C/min from 40 °C to 100 °C, then isothermal for one minute and then cooled down to 0 °C with at rate of 10 °C/min. Finally, the temperature was ramped up to 100 °C with a heating rate of 5 °C/min. The last heating curve was obtained for further analysis.

$^1$H NMR (500 MHz) was used to characterize the copolymer structure. All the samples were dissolved in deuterated chloroform ($\text{CDCl}_3$) at a concentration from 5 mg/mL to 10 mg/mL. Pure PEG and L-lactide samples were also prepared and analyzed.

ElectroForce 3200 from TA Instruments was used to identify the mechanical properties of copolymers. A 20 mm×5 mm×2 mm cuboid-shaped notch was made by laser cutting. The melted copolymers were extruded through plastic syringes into this notch as mechanical test samples. For each type of copolymer, five samples were prepared. All the
samples were cooled down to room temperature in a vacuum oven for at least for 24 hours prior to analysis to ensure the copolymer samples would not degrade and swell by absorbing moisture from the air. Tensile test was performed at a crosshead speed of 5 mm/min.

A Dry Ice Benchtop Freeze Dryer from LABCONCO was used to identify the swelling ratio of copolymers. Three specimens for each type of copolymer with volume around 28 mm$^3$ and mass around 30mg were prepared and placed in a vacuum oven at room temperature a day before. The dry mass of each specimen was measured and marked. All the specimens were immersed in excess PBS solution in vials at the same time point and placed on the shaker plate with slow shake speed in the incubator at 37 °C for 24 hrs. The swollen weight of specimens was measured and then they were frozen in a -20 °C freezer for 48hrs and finally freeze-dried for 24 hrs to obtain the final dry polymer mass.
4.0 Results and Discussion

4.1 $^1$H NMR

Three distinct and intense peaks in the NMR spectrum showed that the PLLA-PEG-PLLA copolymers contain three different hydrogen protons. As shown in Figure 5, PLLA has two types of hydrogen protons and PEG has one type of hydrogen proton. Three peaks of copolymers were characterized. –CH group appeared at 5.2 ppm and 4.3 ppm, -CH$_3$ group appeared from 1.8 ppm to 0.9 ppm, -CH$_2$ group appeared at 3.6 ppm. The results of integration of different peaks were used to calculate the molecular weight of PLLA that was added onto PEG. The chemical shift of the peak at 3.6 ppm could be caused by the connection joint between PLLA and PEG blocks, as the surrounding groups of -CH$_2$ on the copolymers were slightly different than in the homopolymer blocks. The molecular weight of PLLA added onto each PEG was directly calculated by the ratio of –CH and –CH$_2$. The number average molecular weight of PEG was assumed to be the same as labeled. As an example, the copolymer using PEG 20,000 with a high loading of PLLA was calculated by the following equation:

\[
M_{n,copolymer} = M_{n,PEG} + DP_{PLLA} \times M_{r,PLLA}
\]

\[
= 20,000 + 144 \times \frac{1.0259}{10.9133 \times 4} \times \frac{20,000 - 18}{44} = 26147 \ g/mol
\]
Figure 5. (A) Structure of PLLA-PEG-PLLA copolymers; (B) $^1$H-NMR spectrum of 20K; (C) $^1$H-NMR spectrum of 20K-10.64.

The molar ratio and mass ratio of PEG over PLLA can be calculated by the following formulas:
Molar Ratio \( \left( \frac{PEG}{PLLA} \right) = \frac{M_{n,PEG}}{M_{r,PEG}} \cdot \frac{M_{n,PLLA}}{M_{r,PLLA}} \)

Mass Ratio \( \left( \frac{PEG}{PLLA} \right) = Molar Ratio \left( \frac{PEG}{PLLA} \right) \times \frac{M_{r,PEG}}{M_{r,PLLA}} \)

All of the \(^1\text{H} \) NMR results were summarized in Table 1.

Table 1. \(^1\text{H} \) NMR results of synthetic copolymers.

<table>
<thead>
<tr>
<th>Copolymers</th>
<th>( M_n ) (g/mol)</th>
<th>( M_{n,PLLA} ) (g/mol)</th>
<th>Molar Ratio ( \frac{PEG}{PLLA} )</th>
<th>Mass ratio ( \frac{PEG}{PLLA} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG(_{454})</td>
<td>20,000</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLLA(<em>{5.3})-PEG(</em>{454})-PLLA(_{5.3})</td>
<td>21534</td>
<td>1534</td>
<td>42.67</td>
<td>13.04</td>
</tr>
<tr>
<td>PLLA(<em>{11.6})-PEG(</em>{454})-PLLA(_{11.6})</td>
<td>23346</td>
<td>3346</td>
<td>19.54</td>
<td>5.98</td>
</tr>
<tr>
<td>PLLA(<em>{21.3})-PEG(</em>{454})-PLLA(_{21.3})</td>
<td>26147</td>
<td>6147</td>
<td>10.64</td>
<td>3.25</td>
</tr>
<tr>
<td>PLLA(<em>{54.5})-PEG(</em>{454})-PLLA(_{54.5})</td>
<td>37686</td>
<td>15686</td>
<td>4.17</td>
<td>1.27</td>
</tr>
</tbody>
</table>
4.2 GPC

The GPC analysis results of the synthesized copolymers validated the $^1$H NMR results, which proves that the molecular weights of copolymers changed with varied PLLA loading. Detailed information of PLLA-PEG-PLLA copolymers is shown in Table 2.

**Table 2: GPC result of the synthesized copolymers.**

<table>
<thead>
<tr>
<th>Copolymers</th>
<th>$M_n$ (g/mol)</th>
<th>$M_w/Mn$ (PDI)</th>
<th>Molar Ratio PEG/PLLA</th>
<th>Mass ratio PEG/PLLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG$_{453}$</td>
<td>19911 ± 1633</td>
<td>1.10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLLA$<em>{9.4}$-PEG$</em>{453}$-PLLA$_{9.4}$</td>
<td>22620 ± 1358</td>
<td>1.12</td>
<td>24.06</td>
<td>7.35</td>
</tr>
<tr>
<td>PLLA$<em>{8.3}$-PEG$</em>{453}$-PLLA$_{8.3}$</td>
<td>22289 ± 1121</td>
<td>1.14</td>
<td>27.40</td>
<td>8.38</td>
</tr>
<tr>
<td>PLLA$<em>{29.8}$-PEG$</em>{453}$-PLLA$_{29.8}$</td>
<td>28493 ± 1815</td>
<td>1.24</td>
<td>7.59</td>
<td>2.32</td>
</tr>
<tr>
<td>PLLA$_n$-PEG$_m$-PLLA$_n$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

A PEG standard calibration curve was built to characterized the PLLA-PEG-PLLA molecular weight. Samples A and C had significant various additions of PLLA loadings on PEG chains ends with same PEG initiator amount. The addition of PLLA ranged from 2709 g/mol to 8582 g/mol and the degree of polymerization (DP) of PLLA ranged from 9.41 to 29.80 on each side of PEG chains. The polydispersity (PDI) of the synthetic copolymers ranged from 1.10 to 1.24 showing a relatively high uniformity distribution of molecular weights. The degree of polymerization is obtained from the following formula:
\[ DP_{PEG} = \frac{M_n,PEG}{M_r,PEG} \]

\[ DP_{PLLA} = \frac{M_n - M_{n,PEG}}{M_r,PLLA} \]

\( M_{r,PEG} \) represents the molecular weight of PEG repeat units equals to 44, and \( M_{r,PLLA} \) equals to 144. PEG has two hydroxyl groups on the both of the chain ends, so PLLA has even opportunity to be added on each side of PEG chains. In the calculation, the \( DP_{PLLA} \) is divided by 2 to indicate the PLLA was evenly added onto both sides of the PEG chains. PDI demonstrates the distribution of the molecular weights of copolymer. Sample E doesn’t have a GPC result, as the copolymer couldn’t totally be dissolved by the GPC mobile phase, 80 % methanol and 20% water, since \(^1\)H NMR indicates that this copolymer contains more PLLA than others. Samples B and C did not show an increased molecular weight with more PLLA loading, since the molecular weight range of PEG standard for calibration is very large, ranges from 895 g/mol to 62,300 g/mol, and the test range of the columns of the GPC is up to \(10^6\) Da. This could result in a lower resolution for narrower molecular weight changes. Thus, the \(^1\)H NMR results were more reliable than GPC results.

We categorized PLLA-PEG-PLLA copolymers with the molecular weight of PEG and PEG/PLLA molar ratio obtained by \(^1\)H NMR results in the following study. 20K represents P/EG 20,000; 20K-42.67 represents PEG 20,000 with low PLLA loadings; 20K-
19.54 represents PEG 20,000 with medium PLLA loadings; 20K-10.64 represents PEG 20,000 with high PLLA loadings; 20K-4.17 represents PEG 20,000 with extra high PLLA loadings. PEG 8,000 with high PLLA loading studied by Wei et al., 8K-11.7, represents the composition of PEG 8,000 with high PLLA loadings obtained from $^1$H NMR results.

### 4.3 DSC

The thermal properties of the copolymer could be showed by DSC. The materials with an onset melting temperature higher than body temperature would be ideal. The peak melting temperature is important to be considered for a 3D printing extrusion method. The heat of melting indicated the amount of heat per gram of the copolymers needed to be melted. All the copolymers’ DSC results were summarized in Table 3. All the copolymers were tested in triplicate with a heat-cool-heat procedure.

**Table 3: Detailed DSC results of copolymers.**

<table>
<thead>
<tr>
<th>Copolymers$^a$</th>
<th>$T_{m,\text{onset}}$ (°C)</th>
<th>$T_{m,\text{peak}}$ (°C)</th>
<th>$\Delta H_m$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20K</td>
<td>61.32±0.70</td>
<td>67.96±0.14</td>
<td>140.77±3.81</td>
</tr>
<tr>
<td>20K-42.67</td>
<td>55.64±0.57</td>
<td>60.01±0.45</td>
<td>92.56±0.54</td>
</tr>
<tr>
<td>20K-10.64</td>
<td>54.26±0.15</td>
<td>59.18±0.16</td>
<td>76.04±1.28</td>
</tr>
<tr>
<td>20K-4.17</td>
<td>52.06±0.66</td>
<td>57.51±0.48</td>
<td>68.44±1.26</td>
</tr>
<tr>
<td>8K-11.7</td>
<td>49.16±1.02</td>
<td>54.54±0.50</td>
<td>33.30±4.00</td>
</tr>
</tbody>
</table>
According to Anders Sodergard and Mikael Stolt’s study on pure PLLA, PLLA is a polymer with a melting point ($T_m$) about 170 °C [20]. All samples were ramped up to 200 °C at a heating rate of 5 °C/min only once to detect the potential of PLLA homopolymer. However, none of them showed an endothermic peak about 170 °C, which indicates that no PLLA homopolymer was mixed within the synthesized copolymers. This also means that no water was mixed within the synthesized copolymers. The block copolymers only showed one endothermal and PEG-related melting temperature, which means PLLA was evenly added onto PEG.

Figure 6. DSC thermograph of each type of synthetic copolymers.
The copolymers having only one melting peak are great since PEG-PLLA composites were uniformly distributed. Also, as the melting temperature of PEG is lower than PLLA, the copolymers can maintain a relatively lower temperature melting, which enables use for further applications that print with other biomaterials which will degrade at relatively higher temperature. The small endothermal peak which appeared in 20K-4.17 is the crystallization peak. The 10 °C/min cooling rate is too fast for 20K-4.17, as it required more time to crystalline since the entanglements were more tightly bonded than other copolymers. When a 1 °C/min cooling rate out of the figure is conducted, the crystalline peak was completely located in the cooling procedure.

Each copolymer did three repeating samples. All the copolymers only ha one melting peak during the procedure except 20K-4.17. According to R. Majumdar, K. S. Alexander and A. T. Riga’s study on physical characterization of polyethylene glycols by thermal analytical techniques and the effect of molecular weight [23], the melting temperature of PEG is increased with the increasing PEG molecular weight. High molecular weight PEGs showed higher melting temperature due to the higher interactions of PEG chain ends, which will require more energy to be melted.
Figure 7. Onset melting temperature of different copolymers with different PLLA loadings. (A) 20K; (B) 20K-42.67; (C) 20K-19.54; (D) 20K-10.64; (E) 20K-4.17.

The onset melting temperature indicated the lowest temperature of a copolymer required to start to melt. A biomaterial with an onset melting temperature lower than body temperature (37 °C) will start to melt once it contacts the human body. As shown in Figure 7, the black horizontal line indicates the human body temperature and temperatures below this line are not good for further consideration. Obviously, the onset melting temperatures of all the copolymers were higher than body temperature, which ensures they can be potentially considered as implants. However, 20K-42.67 was statically similar with 20K-
19.54. 20K-10.64 and 20K-4.17 were statistically different from all the other copolymers.

Figure 8. Peak melting temperature of different copolymers with different PLLA loadings. (A) 20K; (B) 20K-42.67; (C) 20K-19.54; (D) 20K-10.64; (E) 20K-4.17.

The peak melting temperature is an important element in 3D printing. The 3D printer can be adjusted to a relatively higher temperature than the peak melting temperature to ensure all the material can be melted before printing. With the copolymers that were synthesized, all the copolymers have a relatively lower peak melting temperature. This could allow the extruder to be heated up to a lower temperature before printing which is safer and easier to control. 20K-42.67 are statistically not significantly different with 20K-
19.54, and these two materials can be fully melted under the similar melting temperature which is set up for printer head. With the amount of PLLA loading increasing, the peak melting temperature decreases indicated that the copolymer with higher PLLA loadings can be easier and more safely extruded than with lower PLLA loadings.

Figure 9. Melting enthalpy of 20K with various PLLA loadings. (A) 20K; (B) 20K-42.67; (C) 20K-19.54; (D) 20K-10.64; (E) 20K-4.17.

Figure 9 showed a clear trend that the melting enthalpy decreased with the increasing PLLA loadings, which indicated that the crystallinity of the copolymers decreased with more PLLA side blocks added onto PEG center blocks. In general, PEG with higher molecular weights will have higher crystallinity [19]. The PLLA side blocks added to PEG
center blocks decreased the crystallinity of the copolymer, causing the copolymer to require less energy to be melted. This phenomenon is also proved in Figure 6, where the heat flow also decreased at higher PLLA loadings. In this experiment, all the copolymers containing PLLA loadings from low to extra high were included for further research, since their onset and peak melting temperatures are above 37 °C and the heat of melting are relatively not too high.

### 4.4 Equilibrium swelling study

The hydrophilicity of the synthesized copolymers could be modified by adjusting the ratio of the hydrophilic and hydrophilic segments [8]. For consideration as an implant for nerve guidance channels for nerve repair, the equilibrium swelling ratio of the copolymer at 37 °C is an important consideration to be studied. Equilibrium swelling study provided the data of the amount of liquid the copolymer would contain at the equilibrium state. We selected 8K-36.6, 8K-18 and 8K-11.7 from Wei Wu’s study on PLLA-PEG-PLLA copolymers [6] along with 20K-42.67, 20K-19.54 and 20K-10.64, to be tested. They were loaded into syringes and immersed in an oil bath at 100 °C. The synthesized copolymers were extruded into a prepared rectangular Teflon mold after being melted. After cooldown to room temperature, the samples were made to similar size (28mm³) with rounded shape, and the density of samples ranged from 1.0 g/mm³ to 1.1 g/mm³, which is the common
density range of the polymer. Three repeated samples were prepared for each type of copolymer and put into a vacuum oven overnight. The mass of vials and labels were measured and marked as \( W_{v1} \). Phosphate-buffered saline (PBS) solution was prepared several days before the study and placed at the refrigerator at 4-5 °C. The pH was measured by a VWR Benchtop Meter. Three repeated samples of each type of copolymer were put into scintillation vials with excess PBS solution. Then the vials were placed in an incubator at 37 °C with a shaker plate with a slow spinning rate for 24 hours. The swelled copolymers were carefully placed on weighing paper to get rid of additional PBS solution on the copolymers’ surfaces. Then the swollen mass was measured and marked as \( W_s \). The swollen samples were then frozen in the freezer at -20 °C for 48 hours to ensure all the swelling solution was completely frozen. A lyophilizer was used to lyophilize the frozen samples. The lyophilizer was kept running for 24 hours to guarantee the PBS was entirely removed. The mass of the samples after lyophilization in vials was marked as \( W_{d1} \). \( W_L \) represents the neat mass of each sample after lyophilization. The swelling ratio of copolymers at equilibrium state was calculated with the following equations:

\[
W_L = W_{d1} - W_{v1}
\]

\[
q_{equilibrium} = \frac{W_s - W_L}{W_L}
\]

Samples A- C in Figure 10 were 8K-36.6, 8K-18 and 8K-11.7, respectively, and
samples D-F were 20K-42.67, 20K-19.54 and 20K-10.64, respectively. These samples were swollen for 24 hours. It is obvious that samples 8K-36.6, 8K-18 and 20K-42.67 were cracked after one day of swelling, as the rounded samples were cracked into small pieces. 8K-11.7 had some crevices on the edges and 20K-19.54 had some transverse crevices. 20K-10.64 also had crevices but not as obvious as 20K-19.54. This indicated that the copolymers with the same amount of PEG can imbibe less PBS with longer PLLA blocks. It also showed that with the same amount of PLLA, the increment of the molecular weight of PEG would improve the ability of copolymers to maintain its structure and morphology in PBS solution. By adjusting the mole ratio of hydrophilic PEG and hydrophobic PLLA of the copolymers, the copolymer could have varied swelling behavior. This also matched the $^1\text{HNMR}$ results at Table 1. 8K-36.6, 8K-18 and 20K-42.67 were excluded from further research due to their poor ability to maintain structure in PBS solution.
Figure 10. Prepared copolymers samples after 24 hours swollen at 37°C under slow spinning rate. (A) 8K-36.6; (B) 8K-18; (C) 8K-11.7; (D) 20K-42.67; (E) 20K-19.54; (F) 20K-10.64.

![Swelling ratio graph]

Figure 11. The swelling ratio of samples. From left to right are 8K-11.7; 20K-19.54; 20K-10.64.

4.5 In vitro degradation study

According to Stephanie Deshayes and Andrea M. Kasko’s study of polymeric biomaterials with engineering degradation, biomaterials implanted into the human body should have a controlled degradation behavior and period [21]. An in vitro degradation
study in biological environment was thus needed. PBS solutions were used to mimic the biological environment. Three types of synthesized copolymers, 8K-11.7, 20K-19.54 and 20K-10.64, were studied in this test. Three samples for each type of copolymer were prepared in the same size and shape and cooled to room temperature. Each mass of dried sample was measured and marked as \( W_{d2} \), which separated from \( W_{d1} \) in the swelling study. The mass of vials with labels was measured and marked as \( W_{v2} \), which set apart from \( W_{v1} \) in the swelling study. The prepared samples were immersed in the excess volume PBS solution (5 mL) and placed on the shaker plate with slow spinning rate in the incubator at 37 °C for 24 hours. At the same time point each day, the mass of swollen samples and vials were measured after the excess PBS solution was removed by glass pipettes. The mass of swollen samples and vials was marked as \( W_{s+v_f} \). The swelling ratio, \( q_{\text{swell}} \), of each sample at each day can be calculated by the following equation:

\[
q_{\text{swell}} = \frac{W_{s+v_1} - W_{v_1}}{W_{d2}}
\]

Then the average weight swelling ratio curve of each sample at each day was obtained by PRISM analysis software with standard deviation at each day, and the samples were no longer measured once the structure was totally cracked.
Figure 12. Average weight swelling ratio of each type of synthesized copolymer at each day.

As shown in the Figure 12, all the synthesized copolymers underwent a quick swelling process at day 1. 20K-19.54 swelled the most PBS solution. By comparing with 20K-19.54, 20K-10.64 contained more PLLA side blocks, thus tighter bonds were contained in 20K-10.64. The increasing length of PLLA chains led to a lower swelling ability of the copolymer. The mass ratio from $^1$H NMR also confirmed this. The mass ratio of 20K-10.64 was 3.25, which was less than the mass ratio of 5.98 of 20K-19.54. Per mass, 20K-10.64 contained more PLLA side blocks than 20K-19.54. This caused the lower swelling ratio of 20K-10.64. Copolymers containing longer PEG center blocks and more
PEG repeat units provided the copolymer with the ability to absorb more PBS. Longer PLLA block lengths and more PLLA repeat units supported the copolymer to maintain its structure during degradation. It was obvious that 8K-11.7 was totally degraded after 7 days. 20K-19.54 had a relatively longer degradation time but was still totally degraded at day 8. However, 20K-10.64 could hold its structure and morphology for 13 days due to the longer PLLA block length.

Figure 13. Average mass change of synthesized copolymers at each day.

The average mass change study was also conducted with the existing former data. The mass change of each sample was calculated by the following formula:
\[ W_{\text{change}} = (W_{5+n} - W_{n}) - W_{d2} \]

\( W_{\text{change}} \) represented the mass change of each sample at each day. \( W_{5+n} \), \( W_{n} \), and \( W_{d2} \) represented the same as in the average weight swelling study. All the synthetic copolymers imbibed lots of PBS solution after one day and slowly swelled relatively lower amounts of solution from day 1 to day 4. The same reason mentioned in the swelling ratio study could explain that 20K-10.64 lasted the longest time among the copolymers during the study. All samples were no longer measures after the structure completely cracked.

### 4.6 Mechanical properties

The mechanical tests showed the mechanical properties of the synthesized copolymers. The copolymers with various PLLA side blocks and PEG center blocks would have different mechanical properties. The axial failure test was conducted using an ElectroForce 3200 from TA Instruments. Five repeated specimens for each type of synthesized copolymer were prepared. The copolymers were loaded in plastic syringes and melted in an oil bath at 100 °C. The melted copolymers were extruded into a 10 mm×5 mm×2 mm cuboid shaped Teflon notch prepared by laser cutting after being totally melted. All the redundant parts were cut off after the specimens cooled down to room temperature. The specimens were placed in the vacuum oven overnight to ensure that moisture from the air would not be absorbed by the specimens. The gauge area was 50 mm² (10 mm×5 mm).
and two axial clamps were already prepared at aligned state to ensure no bending force during the test. The engineering stress and engineering strain could be calculated by the following formulas:

\[
\sigma = \frac{F}{A}
\]

\[
\varepsilon = \frac{\Delta L}{L} \times 100\%
\]

F represented the load (N) on the specimens during the test; A represented gauge area, which was 20 mm²; L represented the specimen gauge length (10 mm) of each sample; \( \Delta L \) represented the change in gauge length, which could be directly obtained from the data.

Figure 14. Stress strain curve of each type of representative copolymer.
Stress strain curves of each type of copolymer were plotted by PRISM analysis software. The most representative curve from each type of copolymer from five specimen was selected for plotting. Then a comparative stress strain curve of the five type of copolymers were plotted in Figure 14. The results showed the mechanical properties of the PLLA-PEG-PLLA block copolymers highly depend on the composition. With the increment of PLLA side blocks added onto the same molecular weight PEG center blocks, the specimen could be elongated further and resisted more external force.

Figure 15. Ultimate strain for each type of copolymer.
* indicates a statistical difference between groups as determined by one-way ANOVA.

Figure 16. Ultimate stress for each type of copolymer.

* indicates a statistical difference vs. all other groups as determined by one-way ANOVA.

As shown in figures 15 and 16, the ultimate stress and strain were highly dependent on the copolymer composition. At the same molecular weight of the PEG center block, with longer PLLA side blocks added onto PEG chains, the ultimate stress and strain were
increased. $^1$H NMR results in Table 1 also supported this, since the mass ratio decreased from 13.04 to 1.28, which proved that more PLLA side blocks were added onto the same repeat units of PEG center blocks.

![Graph showing Young's modulus of each type of copolymers.](image)

**Figure 17. Young’s modulus of each type of copolymers.**

* indicates a statistical difference vs. all other groups as determined by one-way ANOVA.

The young’s modulus was also increased with longer PLLA side blocks when PEG molecular weight was consistent. The molar fraction of PEG and PLLA would affect the young’s modulus. The molar fraction of PLLA was increased from 2.34% to 19.34%, this
significant molar fraction difference resulted in the young’s modulus increased with consistent molecular weight of PEG. From Wei Wu’s GPC results of 8K-11.7 [18], the mole fraction of the PEG and PLLA was 94.74 % and 5.26 %. The mole fraction of the PEG and PLLA of the 20K-19.54 copolymer were 95.12 % and 4.88%. The mole fraction of these two copolymers were not significantly different, which was also verified by the one-way ANOVA test. This resulted in these two copolymers having similar young’s modulus. 5.26% PLLA molar fraction in 8K-11.7 copolymer was higher than 2.34% PLLA molar fraction in 20K-42.67, this resulted in the young’s modulus varying between these two copolymers.
Figure 18. Comparison of PLLA block length and Young’s modulus for each type of copolymer.

Figure 18. was plotted for further studying the influence of PLLA side block length to the mechanical properties of copolymers. The trend was similar with the trend of young’s modulus. With more PLLA side blocks onto consistent PEG center blocks, the higher young’s modulus the copolymer would possess. The similar molar fraction of PEG to PLLA also explained the similar young’s modulus of 8K-11.7 and 20K-19.54, although they contain various PLLA block length.
5.0 CONCLUSIONS

The molecular weights and chemical structure of synthetic polymer were characterized by GPC and $^1$H NMR. Various amount of PEG and PLLA block lengths provided the copolymers with different molar ratios. The degree of polymerization, as well as number of repeat units, of PLLA could be controlled by the amount added. The thermal properties of the copolymers were characterized by DSC. Higher PEG/PLLA molar ratio provided the synthetic copolymers with a lower melting point and melting enthalpy. Lower melting enthalpy was preferable, since PLLA-PEG-PLLA copolymers were expected to be extruded with neuron cells and nutrients. Thus, a higher melting point and enthalpy were not ideal since the cells could be inactivated or killed. The introduction of PLLA side blocks onto PEG center blocks drastically influenced the water absorption of the copolymers. Longer PLLA block lengths provided the copolymer with less water absorption. Copolymers with a longer degradation period would be ideal as an implant in the body. Copolymers 20K-42.76, 20K-19.54, 8K-5.2, and 8K-10.6 were excluded, due to the poor ability to maintain their structure in PBS. PLLA-PEG-PLLA acted as a thermoplastic elastomer material, which would flow above the melting point and crystalize below its crystalline point. Since peripheral nerves have a tensile strength of 11.7 MPa [22], 20K-4.17 was the best candidate as a nerve guidance tube due to its tensile strength of ~12 MPa.
6.0 RECOMMENDATIONS

6.1 pH regulation

The synthesis of copolymers with various block lengths of PLLA were successfully polymerized onto PEG center blocks. A preliminary pH test was conducted outside of this paper. Copolymer 8K-11.7 and 20K-10.64 were being immersed into DI water and PBS solution at two separate vials. The pH value versus time were measured. Both copolymers released large amounts of lactic acid over 12 days. The pH of both vials were decreased from 7.4 to 4, which may cause cell death in such an environment. There are two ways to regulate this situation: one is to add non-toxic polymer with amine groups like poly(methyl acrylate) onto the end PLLA groups and another is to replace PEG with polymers with more carbon in the backbone and hydroxyl groups on both ends, like poly(1,4-butylene glycol). However, the biocompatibility of this kind of polymer must be studied.

6.2 Mechanical test at different conditions

The copolymer synthesized showed increasing ultimate stress, strain and tensile strength with decreasing PEG/PLLA molar ratio. The tensile failure test was conducted at room temperature. Although 20K-4.17 showed the closest tensile strength to in situ peripheral nerves, it was not tested at 37 °C nor in an equilibrated hydrated state. Cohn and his co-worker introduced hexamethylene diisocyanate (HDI) as a chain extension agent
into PLA-PEG-PLA, which forms crosslinkers with 1000% elongation at break and the ultimate tensile strength reached up to 30 MPa. The introduction of crosslinker to the copolymer may be an ideal method to improve the mechanical properties.
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