

Alternating block polyurethanes based on PCL and PEG as potential nerve regeneration materials

Guangyao Li, Dandan Li, Yuqing Niu, Tao He, Kevin C. Chen, Kaitian Xu
Multidisciplinary Research Center, Shantou University, Daxue Lu 243, Shantou, Guangdong 515063, China

Received 5 January 2013; revised 19 March 2013; accepted 21 March 2013
Published online 00 Month 2013 in Wiley Online Library (wileyonlinelibrary.com). 10.1002/jbm.a.34732

Abstract: Polyurethanes with regular and controlled block arrangement, i.e., alternating block polyurethanes (abbreviated as PUCL-alt-PEG) based on poly(e-caprolactone) (PCL-diol) and poly(ethylene glycol) (PEG) was prepared via selectively coupling reaction between PCL-diol and diisocyanate end-capped PEG. Chemical structure, molecular weight, distribution, and thermal properties were systematically characterized by FTIR, 1H NMR, GPC, DSC, and TGA. Hydrophilicity was studied by static contact angle of H2O and CH2I2. Film surface was observed by scanning electron microscope (SEM) and atomic force microscopy, and mechanical properties were assessed by universal test machine. Results show that alternating block polyurethanes give higher crystal degree, higher mechanical properties, and more hydrophilic and rougher (deep ravine) surface than their random counterpart, due to regular and controlled structure. Platelet adhesion illustrated that PUCL-alt-PEG has better hemocompatibility and the hemocompatibility was affected significantly by PEG content. Excellent hemocompatibility was obtained with high PEG content. CCK-8 assay and SEM observation revealed much better cell compatibility of fibroblast L929 and rat glial cells on the alternating block polyurethanes than that on random counterpart. Alternating block polyurethane PUCL20-E4 with optimized composition, mechanical, surface properties, hemocompatibility, and highest cell growth and proliferation was achieved for potential use in nerve regeneration. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 00A:000–000, 2013.

Key Words: alternating block polyurethanes, poly(e-caprolactone), poly(ethylene glycol), biocompatibility, rat glial cell

How to cite this article: Li G, Li D, Niu Y, He T, Chen KC, Xu K. 2013. Alternating block polyurethanes based on PCL and PEG as potential nerve regeneration materials. J Biomed Mater Res Part A 2013:00:000–000.

INTRODUCTION

Biodegradable block polyurethanes have been used in biomedical devices for many years due to their excellent mechanical, processing properties, and adequate biocompatibility.1–3 Advantages of block polyurethanes over other biomaterials lie in that their block and phase domain structures can be easily controlled over a considerable range through selecting appropriate structure and proportion of hard and soft block.4 Many kinds of biodegradable polyurethanes were synthesized by incorporating different biodegradable block, such as polyhydroxalkanoates (PHAs)5–7 and polycaprolactone (PCL),8–10 into backbone of polyurethanes. Poly(e-caprolactone) (PCL), as a biodegradable and biocompatible aliphatic polyester with good mechanical, thermoplastic properties, and nontoxicity, has been widely used in biomedical applications.11 Degradation product of PCL is 6-hydroxyhexanoic acid, which is natural in the human body.12 Poly(ethylene glycol) (PEG) is widely used in colloidal drug delivery system and other hemocompatible biomaterial because it is uncharged, hydrophilic, and nonimmunogenic.13,14 It is nontoxic and FDA-approved for human intravenous, oral, and dermal applications. Development of PCL related materials from diblock and triblock copolymers to multiblock copolymers have received attention for their potential applications,15–17 in which novel biodegradable segmented polyurethanes with a wide range of physical properties and adjustable biocompatibility have been developed for scaffolds, sutures, fixation devices, vascular grafts, and other tissue engineering. Recently, our lab has achieved a new type of block polyurethane, i.e. alternating block polyurethanes.5,18,19 These materials possess well controlled and determined chemical structure as well as regular microstructure. The regular structures endow materials with more special and intriguing properties, such as better biocompatibility, mechanical, and shape forming properties, giving us capacity for more sophisticated applications. The exciting achievements stimulate us to develop alternating block polyurethanes of PCL and PEG as novel biomaterials. Results herein are reported.

EXPERIMENTAL PROCEDURE

Materials

PCL (Guanghua Weiye, Guangdong, China), poly(ethylene glycol) (PEG, Sigma-Aldrich), 1,6-hexamethylene diisocyanate (HDI, Alfa-Aesar), and stannous octanoate (Alfa-Aesar) were...
used as received. Toluene-p-sulfonic acid, chloroform, 1,4-butandediol, 1,2-dichloroethane, dichloromethane, petroleum ether, ethylene glycol, methanol were AR grade, purchased from Guanghua (Guangdong, China) and used as received. PCL-diol prepolymer was prepared via acid catalyzed alcoholysis as reported procedure.19

**Preparation of PEG-diisocyanate prepolymer**

Diisocyanate terminated PEG was prepared according to modified procedure.5 Typically, 0.8 g PEG (Mn = 400, 2 × 10⁻³ mol) was dissolved in 10 mL 1,2-dichloroethane in a 50 mL two-neck flask at 100°C. Then, any trace of water in the system was removed through azeotropic distillation with only 4 mL of 1,2-dichloroethane left in the flask. The 4 mL remaining solution and two drops of stannous octanoate (≈5 × 10⁻³ g) was transferred into a 5 mL syringe. This solution was added dropwise into a 100 mL four-neck flask in which 0.75 g HDI (4.4 × 10⁻³ mol) and 10 mL 1,2-dichloroethane were placed in advance. The reaction was carried out at 50°C for 5 h under a nitrogen atmosphere; followed by completely removing the solvent and excessive HDI under vacuum. Remained PEG-diisocyanate was kept in the flask and used for next reaction directly.

**Preparation of PCL and PEG based alternating block polyurethanes**

Amount 0.002 mol PCL-diol was dissolved in 30 mL 1,2-dichloroethane. The moisture was removed by azeotropic distillation. The remained 15 mL solution was transferred into a 25 mL injector and dropped slowly into the flask of PEG-diisocyanate in 20 mL 1,2-dichloroethane from above step. After 48 h reaction at 75°C, the product was cooled to room temperature and precipitated in a mixture of petroleum ether and methanol (20/1, v/v). The precipitate was redissolved in 40 mL 1,2-dichloroethane and was filtrated to remove insoluble substance. To eliminate stannous octanoate residue and possible remaining oligomers, the filtrate was precipitated again in mixture of petroleum ester and methanol. Product was collected and dried under vacuum to constant weight at 40°C. The average yield was 80%.

**Preparation of PCL and PEG based random block polyurethanes**

PUCl-ran-PEG was synthesized from PCL-diol and PEG using HDI as a coupling reagent. Amount of HDI added was equivalent to —OH group in the solution. Typically, 0.001 mol PCL-diol and 0.001 mol PEG dissolved in 20 mL 1,2-dichloroethane in a 100 mL four-neck flask and water was removed by azeotropic distillation with 10 mL solution remained in the flask. When the flask was cooled to 75°C, two drops of stannous octanoate and 0.002 mol HDI were added sequentially. The reaction mixture was stirred at 75°C under a nitrogen atmosphere for 48 h. Purification procedure was the same as process of PUCl-alt-PEG purification.

**Characterizations**

Fourier transform infrared spectra was measured by Nicolet IR200 (Thermo electron) spectrometer. 1H NMR spectrum was measured by a Bruker AV400 NMR spectrometer (Bruker, Switzerland). Molecular weights and the distribution were estimated by gel permeation chromatography (GPC) with a combination of a Waters 1525 pump (Waters), three columns of Shodex GPC K-800 series and a 2414 differential refractive index detector and a UV detector: Differential scanning calorimetry (DSC) was performed on a TA-Q100 calorimeter (TA, TX). The static angle was measured by a sessile drop method at room temperature using a contact angle analyzer (SL600, Solon Information Technology, Shanghai, China). All the sample preparation, determination procedure and conditions were same as our previous procedures.19

Scanning electron microscopy (SEM, JSM-6360 LA, Nikon, Japan) was used for film surface observation. Film samples were mounted on aluminum stumps coated with gold in a sputtering device (FC-1600, Japan) for 1.5 min at 10 mA. Atomic force microscopy (AFM) (MultiMode Nanoscope, Digital Instruments) observation was carried out under ambient conditions using the tapping mode. Height and phase images were recorded simultaneously at a scan rate of 0.8 Hz. The AFM tips (PPP-NCH, Nanoscience Instruments) used had a typical radius of 7 nm or less, and the cantilevers had a resonance frequency of 204–497 kHz. Height and width of the observed lamellar structures were measured from height images, and three measurements were averaged with each sample.

Mechanical properties were tested by universal testing machine (CMT 4204 Sans, Shenzhen, China). Strip-shaped films (thickness 0.1–0.2 mm, width 10 mm) were used. The tensile strength, Young’s modulus and elongation at break were determined at room temperature with an extension rate of 5 mm/min. At least five sample determinations were conducted for the average value.

**Hemocompatibility**

A certain amount of rabbit blood extracted from an animated rabbit mixed with 3.8% sodium citrate solution (ratio: 9/1, v/v) was centrifuged at 1500 rpm for 15 min at 4°C to obtain platelet-rich plasma (PRP). Materials films in glass dish were sterilized with 75% ethanol, washed three times with PBS and equilibrated in PBS for 1 h. Then 1 mL PRP was placed on tested films at 37°C for 1 h after removing the PBS solution. After 1 h incubation, the films were dipped in 2.5% glutaraldehyde buffer solution overnight. Hence, they were dehydrated in an ethanol-gradient series (30, 50, 70, 80, 90, 95, and 100%) for 15 min each, respectively, and were dried under vacuum. The morphologies of platelet adhered on the film surfaces were studied by SEM.

**Immunocytochemistry**

Immunocytochemistry was performed to identify the isolated glial cells.20 Briefly, 1 × 10³/well cells were seeded in 96-well plates and cultured with DMEM plus 20% FBS. After 24 h incubation, cells were washed twice with phosphate buffered saline and fixed in 4% paraformaldehyde for 30 min. After fixation, the cells were washed with PBS twice and then treated with 0.3% Triton X-100 for 15 min. Cell cultures were firstly treated with mouse antiGFAP, as the primary antibody that was diluted in 0.1M FBS solution dissolving 5% normal goat serum.
(1:100, v/v), for 4 h at room temperature. Cultures were then incubated with secondary antibody, rabbit antimouse FITC (exited at 488 nm and emitted green fluorescence) that was diluted in 0.1 M PBS plus 0.3% Triton X-100 (1:30, v/v), for 2 h at room temperature. After washing the culture twice with PBS, digital images (fluorescence inverted microscope Nikon, Elipse TE 2000, Japan) of immunoreactivity were obtained.

**Cell culture**

Mouse fibroblast L929 cell and rat glial cell were kindly provided by Lab of Neuroscience (Shantou University, Shantou, China). Cells were cultured in DMEM medium (gibco) supplemented with 10% FBS (L929 cell), 20% FBS (glial cell), 100 U/mL penicillin (Sigma) and 100 U/mL streptomycin (Sigma) in a CO₂ incubator (forma 3111, US) supplied with 5% CO₂ at 37°C. Confluent cells were digested using 0.25% trypsin-0.02% EDTA, followed by centrifugation (1000 rpm for 3 min) to harvest the cells. Subsequently, the single cell suspension was used for cell number calculation using hemacytometer $1.0 \times 10^4$ cells in each film coated dish. Cultivation was conducted for 24, 48, and 72 h. Then, CCK-8 assay was used for cell viability study while SEM was used for cell morphology observation.

**Cell viability**

A cell count kit-8 (CCK-8, Beyotime, China) was employed in the experiment to quantitatively evaluate cell viability. After 24, 48, and 72 h incubation, culture medium was removed and the culture was washed with PBS twice. Approximately 450 mL serum-free DMEM medium and 50 mL CCK-8 solution were added to each sample, followed by incubation at 37°C for 3 h to form water dissoluble formazan. Supernatant was transferred to 96-well plate, the optical density (OD) at 450 and 630 nm was determined using a microplate reader (Multiskan MK3, Thermo Labsystems, Finland), 6 parallel experimental groups in each sample were used to assess the cell viability. DMEM containing 10% FBS and CCK-8 for L929 cell or DMEM containing 20% FBS and CCK-8 for rat glial cell was taken as a control.

**Cell morphology**

After 72 h cultivation, cell-seeded films were washed twice with PBS, then immersed in PBS with 2.5% glutaraldehyde (Alfa-Aesar) overnight at 4°C. Subsequently, cell-seeded films were dehydrated in an ethanol-gradient series (30, 50, 70, 90, 95, 100%) for 15 min each. The dehydrated cell-seeded films were dried via lyophilization and then used for SEM observation.

**RESULTS**

**Preparation and characterizations of PUCL-alt-PEG and PUCL-ran-PEG**

PUCL-alt-PEG was prepared from PCL-diol and PEG-diisocyanate prepolymers by solution polymerization (Fig. 1).
The synthesized PCL-diol was characterized by $^1$H NMR as shown in Figure 2. The methylene proton signals of $-\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$ group from PCL are assigned at $\delta = 4.06$, $2.31$, $1.65$, and $1.40$ ppm. The proton signal at $\delta = 3.65$ ppm is associated with terminated unit $-\text{OCH}_2\text{CH}_2\text{CH}_2\text{OH}$ and $-\text{OCCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$, which deprives from 1,4-butanediol and PCL, respectively. Molecular weight of PCL-diol was calculated by $^1$H NMR integration through equation below:

$$M_n = M_{CL} \times \frac{2N}{P} + M_{BD}$$

(1)

$M_{CL}$ and $M_{BD}$ represent molecular weight of CL unit and 1,4-butanediol, respectively; $N$ and $2/P$ are the integration of methylene ($-\text{OCH}_2-$) from CL ($\delta = 4.06$ ppm) and methylene (HOC$\text{H}_2$-) from 1,4-butanediol ($\delta = 3.65$ ppm).

Synthesis of HDI end-capped PEG ($M_w = 400$) was monitored through FTIR and $^1$H NMR analysis. Figure 3 shows the FTIR spectra, in which $\text{OH}$ absorption of PEG end-groups becomes weaker with reaction time. As the reaction runs for $2$ h, $\text{OH}$ absorption at $3522$ cm$^{-1}$ disappears [Fig. 3(b)] and new absorption belonged to urethane linkage at $3338$ cm$^{-1}$ emerges [Fig. 3(c)], indicating the telechelic prepolymer PEG-diisocyanate was successfully prepared.

$^1$H NMR analysis was also used for tracking the synthesis of PEG-diisocyanate (Fig. 4). The imino group is clearly presented at $\delta = 5.0$ ppm while methylene beside ester group $-\text{COO}-\text{CH}_2-$ appears at $\delta = 4.21$ ppm. The PEG-diisocyanate was not successfully produced until the proton integration at $\delta = 3.32$ ppm was equal to proton integration at $\delta = 3.17$ ppm and these two proton integrations became invariant with the extension of time. By comparing Figure 4(a,b), it is clearly seen that the two protons are satisfied with the two conditions as mentioned above as the reaction undergoes for $2$ h. Therefore, it is concluded that the telechelic prepolymer HDI end-capped PEG is successfully prepared after $2$ h reaction, which well correlates with results of FTIR. The obtained PEG-diisocyanate is then reacted at $75^\circ\text{C}$ for $48$ h with PCL-diol to form alternating block polyurethanes (PUCL-alt-PEG) via a selectively end group coupling reaction (Fig. 1). The PCL and PEG based random block polyurethanes, i.e. PUCL-ran-PEG were synthesized with equivalent PCL-diol and PEG-diol using HDI as coupling agent via solution polymerization. The reaction was implemented at $75^\circ\text{C}$ for $48$ h, which was similar to the reaction conditions of PUCL-alt-PEG synthesis.

Chemical structures of both alternating and random block polyurethane were characterized. Representative FTIR spectra of PCL-diol, PUCL-alt-PEG (PUC20-a-E4) and PUCL-ran-PEG (PUC20-r-E4) are compared in Figure 5. Here sample name abbreviation PUC20-a-E4 or PUC20-r-E4 represents U: block polyurethane, a: alternating; r: random; C20
and E4: PCL-diol \((M_n = 2000)\) and PEG \((M_n = 400)\), respectively.

Typical \(^1\)H NMR spectrum of PUCL-alt-PEG sample PUC20-a-E4 is presented in Figure 6. The random series give the identical NMR spectrum (Supporting Information). All the proton signals belong to PCL and PEG segment and urethane links could be clearly confirmed. In addition, the compositions of copolymer could be calculated by integration of PCL and PEG segments, in which both the alternating block polyurethanes and random block polyurethanes meet a satisfactory agreement with the feed ratio (Table I). This agreement in product and feed ratio was further confirmed by splitting peaks of imino group –OOCNH– at \(\delta = 4.94\) ppm and \(\delta = 4.75\) ppm, where the former connected to PEG segment and the latter connected to PCL.\(^5\)

Molecular weight and distribution were determined by GPC and summarized in Table I. Typical GPC chromatographs of samples PUC20-a-E4 and PUC20-r-E4 are shown in Figure 7. The alternating block polyurethanes possess molecular weight of \(9.2-14.0 \times 10^4\) and distribution of \(1.4-1.5\), while the random block polyurethanes have molecular weight of \(8.4-10.0 \times 10^4\) and distribution of \(1.5-1.6\) (Table I). It is interesting that the alternating block polyurethanes have higher molecular weight and lower distribution than the random counterparts yet.

### Table I. Preparation of PCL and PEG Based Block Polyurethanes

<table>
<thead>
<tr>
<th>Series</th>
<th>Sample(^a)</th>
<th>(R_b)</th>
<th>(R_c)</th>
<th>(M_w) (d)</th>
<th>(PDI)</th>
<th>(M_w/M_n) (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUCL-alt-PEG</td>
<td>PUC10-a-E4</td>
<td>1.00</td>
<td>1.03</td>
<td>99,900</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PUC20-a-E4</td>
<td>1.00</td>
<td>0.99</td>
<td>140,600</td>
<td>1.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PUC20-a-E10</td>
<td>1.00</td>
<td>1.01</td>
<td>92,200</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>PUCL-ran-PEG</td>
<td>PUC10-r-E4</td>
<td>1.00</td>
<td>0.98</td>
<td>105,300</td>
<td>1.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PUC20-r-E4</td>
<td>1.00</td>
<td>1.05</td>
<td>96,100</td>
<td>1.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PUC20-r-E10</td>
<td>1.00</td>
<td>0.99</td>
<td>84,100</td>
<td>1.56</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Sample abbreviation PUC10-a-E4 or PUC10-r-E4 represents U: block polyurethane; a: alternating; r: random; C10 and E4: PCL-diol \((M_n = 1000)\) and PEG \((M_n = 400)\), respectively; same for other tables and text.

\(^b\) PEG /PCL molar ratio in feed.

\(^c\) PEG /PCL molar ratio in product determined by \(^1\)H NMR.

\(^d\) Determined by GPC in CHCl\(_3\).

### Thermal properties

Thermal properties of the polyurethanes and their prepolymers are given in Table II. The polyurethanes with crystallinity degree \(X_c\) from none to 30.6% mean the materials are from amorphous to semicrystal. Striking difference between alternating and random block polyurethanes is that alternating block polyurethanes possess a higher crystallinity degree than the random counterparts. Especially at the short chain length of PCL and PEG segments, alternating PUC10-a-E4 exhibits crystallization degree of \(X_c = 20.9\%\), while random PUC10-r-E4 with no crystallization, but amorphous state. All the polyurethanes with a decomposition temperature \(T_d\) up to 300 \(^\circ\)C exhibit enough thermal stability for biomedical purposes.

### Mechanical property

Mechanical properties of the polyurethane samples, including tensile strength \((\sigma_t)\), yield strength \((\sigma_y)\), Young’s modulus \((E)\), and elongation at break \((\varepsilon)\) are listed in Table III and stress–strain curves are given in Figure 8. Among these samples, polyurethanes with higher PCL content, higher crystal degree and urethane linkage such as PUC20-a-E4, PUC20-r-E4 give higher strength, modulus, and even break elongation. The high elongation at break and relatively low modulus value mean that the polyurethanes are soft thermoplastic elastomers.

### Surface property

Static contact angle and surface energy results are summarized in Table IV. It can be seen that due to the inclusion of
PEG segment in the backbone, obtained polyurethanes exhibit lower contact angle hence more hydrophilic than raw materials PCL. With increase of PEG content, the surface of polyurethanes becomes more hydrophilic. However, by varying the length of PCL and PEG segment, it is found that PUC10-a-E4 with PEG content of 23.0% has lower contact angle and hence more hydrophilic than PUC20-a-E10 with PEG content of 30.0%. This phenomenon was also observed in the random block polyurethanes.

Surface morphology of the obtained polyurethane films prepared by solution casting was investigated by SEM and AFM. Typical SEM photos of both types of polyurethanes are given in Figure 9. And a ravine surface with regular pattern appears. The roughness of the surface was further investigated by AFM. Height images of two types of polyurethanes with same chemical composition are presented in Figure 10. Parameters $R_a$ and $R_{\text{max}}$ are both important index for evaluating the roughness degree of surface. It was found that alternating PUC10-a-E4 with $R_a$ (101.8 nm) and $R_{\text{max}}$ (762.9 nm) is much higher than its random counterpart PUC10-r-E4 with $R_a$ (47.6 nm) and $R_{\text{max}}$ (387.9 nm).

Platelet adhesion
As shown in Figure 11, platelets adhered on PCL and PLA films show many extended pseudopods, indicating that all the platelets are activated and blood coagulation would be easily induced. By comparing with PCL and PLA, on the synthesized PU film surface, much less adherent platelets with no obvious pseudopods are observed, suggesting a weak attachment or even only a physical precipitation of platelets on the surface.

Identification of rat glial cells
Glia fibrillary acidic protein (GFAP), a representative protein in glial cell, was selected as a maker for identification of isolated glial cells. Random images are obtained and it is obviously observed that the cellular structure is covered with well-defined green fluorescence representing the GFAP containing phenotype shown in Figure 12. All these phenomena indicated that the isolated neural cells are glial cells.

Mouse fibroblast cell (L929) culture
CCK-8 assay of fibroblast L929 cell cultured on different PU films are given in Figure 13. All the tested films exhibit good cell compatibility. Cell viability of alternating block

### TABLE II. Thermal Properties of PCL and PEG Based Block Polyurethanes

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_g$ (°C)</th>
<th>$T_c$ (°C)</th>
<th>$\Delta H_m^c$ (J/g)</th>
<th>$T_m^d$ (°C)</th>
<th>$\Delta H_m^e$ (J/g)</th>
<th>$X_{\text{PCL}}$ (%)</th>
<th>$T_d^g$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-diol$^h$</td>
<td>27.5</td>
<td>16.1</td>
<td>73.3</td>
<td>51.5</td>
<td>77.3</td>
<td>56.9</td>
<td>319.0</td>
</tr>
<tr>
<td>PEG$^h$</td>
<td>68.3</td>
<td>29.0</td>
<td>30.8</td>
<td>6.8</td>
<td>34.1</td>
<td>–</td>
<td>317.9</td>
</tr>
<tr>
<td>PUC10-a-E4</td>
<td>52.0</td>
<td>–</td>
<td>–</td>
<td>24.7</td>
<td>18.1</td>
<td>20.9</td>
<td>301.1</td>
</tr>
<tr>
<td>PUC20-a-E4</td>
<td>47.4</td>
<td>19.8</td>
<td>23.2</td>
<td>45.1</td>
<td>32.4</td>
<td>30.6</td>
<td>296.7</td>
</tr>
<tr>
<td>PUC20-a-E10</td>
<td>54.8</td>
<td>21.1</td>
<td>16.5</td>
<td>46.4</td>
<td>23.2</td>
<td>27.0</td>
<td>308.0</td>
</tr>
<tr>
<td>PUC10-r-E4</td>
<td>49.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>295.9</td>
</tr>
<tr>
<td>PUC20-r-E4</td>
<td>48.9</td>
<td>24.1</td>
<td>18.3</td>
<td>45.4</td>
<td>27.3</td>
<td>25.9</td>
<td>296.2</td>
</tr>
<tr>
<td>PUC20-r-E10</td>
<td>56.0</td>
<td>18.7</td>
<td>17.1</td>
<td>44.4</td>
<td>21.0</td>
<td>24.4</td>
<td>302.3</td>
</tr>
</tbody>
</table>

$^a$ Glass transition temperature detected from DSC first heating run.

$^b$ Crystallization temperature detected from DSC.

$^c$ Crystallization enthalpy of PCL was determined from DSC.

$^d$ Melting temperature was determined from DSC first heating run.

$^e$ Melting enthalpy of PCL was calculated from DSC first heating run.

$^f$ Crystallinity degree $X_c$ of PCL was calculated from the melting enthalpy ($\Delta H_m$) from DSC first heating run. Reference value $\Delta H_m^0$ of completely crystallized PCL was 136 J/g and $X_c$ (%) $= 100 \times \Delta H_m/W \cdot \Delta H_m^0$, $W$ is the weight fraction of PCL in the copolymer.

$^g$ Decomposition temperature was determined from TGA with 10% weight loss.

$^h$ The molecular weight of PCL and PEG is 2000 and 400, respectively.

### TABLE III. Mechanical Properties of PCL and PEG Based Block Polyurethanes

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\sigma_t$ (MPa)$^a$</th>
<th>$\sigma_y$ (MPa)$^b$</th>
<th>$E$ (MPa)$^c$</th>
<th>$\epsilon$ (%)$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUC20-a-E4</td>
<td>7.9</td>
<td>3.3</td>
<td>45.2</td>
<td>1352.5</td>
</tr>
<tr>
<td>PUC20-a-E10</td>
<td>5.9</td>
<td>2.2</td>
<td>30.9</td>
<td>750.9</td>
</tr>
<tr>
<td>PUC20-r-E4</td>
<td>7.0</td>
<td>3.0</td>
<td>43.9</td>
<td>545.7</td>
</tr>
<tr>
<td>PUC20-r-E10</td>
<td>4.5</td>
<td>2.4</td>
<td>41.5</td>
<td>552.4</td>
</tr>
</tbody>
</table>

$^a$ $\sigma_t$: tensile strength.

$^b$ $\sigma_y$: yield strength.

$^c$ $E$: Young’s modulus.

$^d$ $\epsilon$: elongation at break.

FIGURE 8. Stress-strain diagrams of PCL and PEG based block polyurethanes.
FIGURE 9. SEM images of PCL and PEG based block polyurethanes (×1000, left) and ×4000, right): (a1-a2) PUC10-a-E4; (b1-b2) PUC20-a-E10; (c1-c2) PUC10-r-E4; (d1-d2) PUC20-r-E10.
polyurethane (PUCL-alt-PEG) is obvious higher than the random counterpart PUCL-ran-PEG, mainly due to the surface difference. PUCL-alt-PEG is more conductive to cell attachment and gives high growth rate. OD value on the third day is 36–240% higher than that on first day, especially on PUC20-a-E4 films.

Rat glial cells culture

Figure 14 gives viability of rat glial cell on alternating and random block polyurethane films. Similar to L929 culture, among all the tested films, PUCL-alt-PEG exhibits much better glial cell growth as compared to PUCL-ran-PEG. On the first day, CCK-8 assay gives OD value of PUCL-alt-PEG films almost 200% higher than that of PUCL-ran-PEG films, and further up to about 250% higher than that on the third day culture. As the water contact angles of both polyurethanes are some similar (Table IV), the difference on cell viability would be mainly due to the difference of surface roughness and patterned microstructure between the two types of polyurethanes. Because of moderately hydrophilic surface, PUC20-a-E4 and PUC20-r-E4 with water contact angles of 88.9° and 88.3°, respectively, display the highest value of 1.32 and 0.48, the highest among their own series. It might hint that water contact angle 88° would be suitable wettability for glial cell growth. Among these polyurethanes, PUCL-alt-PEG exhibit much higher OD value at 24 h incubation, suggesting initial cell attachment is more favorable on surface of alternating polyurethanes. This would be attributed to differences on surface topography of both polyurethanes.22 At following days of incubation, mitochondrial activities of glial cell had a remarkable growth on PUCL-alt-PEG surface. This demonstrated that the cell attachment and proliferation and extensive migration take place on PUCL-alt-PEG surface. PU3/4HB-ran-PEG, however, gives low CCK-8 values and performs anomaly change, indicating glial cells are in poor attachment and proliferation.

From above discussion, it is further noted that alternating block polyurethane PUC20-a-E4 displays the highest cell viability of both fibroblast L929 and rat glial cell. This suggests that PUC20-a-E4 be just the polyurethanes with

FIGURE 10. AFM images of (a) alternating block polyurethane PUC10-a-E4; (b) random block polyurethane PUC10-r-E4. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
optimum composition and proper surface properties for cell compatibility. Similar optimum materials composition for cell growth was also observed in our previous research on rat aortic smooth muscle cells (RaSMCs).23

Figure 15 gives a morphology comparison of glial cell on both alternating and random polyurethane films. After 72 h incubation, glial cells spread well and exhibit their original morphology of star-shape on the alternating series PUCL-alt-PEG. However, on random series PUCL-ran-PEG, the cells tend to be fibroid and even some cells are still not in star-shape.

Figure 16 displays SEM image of rat glial cell cultured at 24, 48, and 72 h on alternating polyurethane PUC20-a-E4 film. PCL film, typical materials for nerve regeneration,24 was used as control. It shows that the cells were attached and proliferated greatly with normal flattened appearance. At 24 h incubation, most cells grew into fibrous morphology on the polyurethane film; however, still in round spherical state on PCL film. After 48 h incubation, cells were still in proliferative state and could be observed with many obvious tentacles. After 72 h incubation, all cells on a PUC20-a-E4 film grew into normal flattened appearance with slightly fibrous and had a large number of proliferations to completely cover the surface. On PCL film, however, there were still some cells in spindle or spherical state.

DISCUSSION

Materials characterizations

The formation of PUCL-alt-PEG and PUCL-ran-PEG [Fig. 5(b,c)] was confirmed from that −OH absorption at 3500 cm⁻¹ related to PCL-diol disappears, and new absorptions at 3370, 1530 cm⁻¹ belonging to −NH− unit in urethane linkage emerged. However, no obvious FTIR absorption difference between alternating and random block polyurethane
was seen. The unimodal GPC peak reveals no prepolymer residuals remained in the polyurethane samples (Fig. 7). Higher crystal degree demonstrates alternating block polyurethanes possess more regular microstructure (Table II).

It is also noted that alternating block polyurethanes display higher tensile strength and elongation than random block polyurethanes (Table III), which may be due to higher crystal degree, higher molecular weight, and lower distribution index. Alternating block arrangement would also lead to better phase separation in polyurethanes. The better crystallized stiff and immobile urethane hard segment domains serve as crosslinks between the flexible soft PEG segment domains. This would thus enhance the elastic mechanical properties.

**Surface property**

More hydrophilic surface of shorter segment polyurethanes would be that the shorter segment gives more hydrophilic urethane linkage and thus endows materials more hydrophilicity. It is clearly seen that the alternating series has lower contact angle hence more hydrophilic surface and higher surface energy than random series (Table IV). This phenomenon was also observed in previous study. The reason might be that alternating structure enhances phase separation and more PEG located on surface, even rougher and regular patterned surface.

The alternating PUC10-α-E4 displays a higher ravine pattern and regular rougher surface than random PUC10-r-E4 (Figs. 9 and 10). This could be due to higher crystal degree from alternating block, the more regular architecture as discussed above. The difference would affect its biocompatibility. Higher ravine surface would be favorable for adhesion and growth of some cells such as fibroblast and neurocells.

**Biocompatibility**

Good hemocompatibility of the polyurethanes may be due to their improved hydrophilicity, flexibility of PEG chains, and surface patterned microstructure. The tendency for platelet aggregation further decreases with increasing PEG content and therefore hydrophilicity, as more hydrophilic surface would inhibit platelet adhesion (Fig. 11). With PEG content up to 25.5%, both the alternating and random block polyurethanes exhibited nonplatelet adhesion, demonstrating excellent hemocompatibility. It is also noted that there are obvious less platelets on PUCL-alt-PEG films than that on random block PUCL-ran-PEG. The reason for better hemocompatibility would be due to higher crystal degree and hydrophilicity, more PEG aggregation on surface of the alternating series due to better phase separation.

**FIGURE 12.** Image of immunocytochemistry of rat glial cells. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**FIGURE 13.** CCK-8 assays of fibroblast L929 viability on PCL and PEG based block polyurethane films at 24, 48, 72 h incubation.

**FIGURE 14.** CCK-8 assays of glial cell viability on PCL and PEG based block polyurethane films at 24, 48, 72 h incubation.
Because the cell membrane is phospholipid bilayer structure, appropriate hydrophilic surface will be more conductive to cell initial attachment as reported in our previous research.\(^7\) Compared with water contact angle in Table IV, this would explain that samples PUC10-a-E4 and PUC20-a-E10 give higher optical values than PUC20-a-E4 at 24 h incubation (Fig. 13). However, our previous research also showed that a little more hydrophobic surface would be more suitable for growth and proliferation of some cells such as L929 on the later stage. It would be the reason that PUC20-a-E4 gives the highest growth rate from 24 to 72 h. Further more, surface roughness is also an important factor.\(^{22}\) From SEM and AFM observation, we know that PUCL-alt-PEG displays deeper and even more regular ravine surface than PUCL-ran-PEG (Figs. 9 and 10). This would be the important aspect for much better L929 cell compatibility on PUCL-alt-PEG films. Other types of alternating block polyurethanes also displayed better L929 cell viability than random counterparts did.\(^{18}\) Culture of both L929 and glial cells indicate that alternating PUCL-alt-PEG possesses better cell adhesion, growth, reproduction, and cell morphology than its random counterpart PUCL-ran-PEG (Figs. 13–15).

These results conclude that alternating block polyurethanes are more favorable to sorts of cell attachment, growth, and proliferation, which makes them better candidates in a wide range of biomedical applications. The studied rat glial cell is a crucial cell not only to connect and support nerve components but also to provide growth factor and distributes nutrients in nerve system. The alternating block polyurethanes especially sample PUC20-a-E4 would be important biomaterials in area of peripheral nerve repair. Our initial in vitro degradation test shows that sample PUC20-a-E4 exhibits obvious degradation in phosphate buffer solution after 4 months. This would satisfy the nerve regeneration period.

CONCLUSIONS

Alternating block polyurethanes with regular and controlled structure based on PCL and PEG were prepared. The novel materials possess higher crystal degree, higher mechanical properties, more hydrophilic, and rougher (deep ravine) surface than traditional random block polyurethanes, due to the former regular and controlled structure. The platelet adhesion assay revealed that both type polyurethanes have excellent hemocompatibility at higher content of PEG. But better hemocompatibility for alternating series can be visualized. According to cell culture assay, kinds of cells (i.e., fibroblast L929 and rat glial cells) are more favorable for the attachment and proliferation on surface of alternating block polyurethanes. Among all the tested samples,
alternating block polyurethane PUC20-a-E4 gives not only suitable mechanical properties and hemocompatibility, but also the best cell compatibility, much better attachment, growth, and proliferation of rat glial cell than other polyurethanes and PCL films. This would make the materials highly potential in nerve regeneration.

**TABLE IV. Contact Angle and Surface Energy of PCL and PEG Based Block Polyurethanes**

<table>
<thead>
<tr>
<th>Sample</th>
<th>W_{PEG}</th>
<th>$\theta_{H_2O}$ (°)</th>
<th>$\theta_{CH_2I_2}$ (°)</th>
<th>$r_s^d$</th>
<th>$r_s^p$</th>
<th>($r_s^d + r_s^p$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>–</td>
<td>84.3 ± 0.7</td>
<td>35.4 ± 1.2</td>
<td>35.1 ± 0.5</td>
<td>7.4 ± 1.0</td>
<td>42.5 ± 0.6</td>
</tr>
<tr>
<td>PCL</td>
<td>–</td>
<td>89.1 ± 1.3</td>
<td>37.5 ± 0.9</td>
<td>36.0 ± 1.2</td>
<td>5.3 ± 0.7</td>
<td>41.3 ± 0.8</td>
</tr>
<tr>
<td>PUC10-a-E4</td>
<td>23.0</td>
<td>67.2 ± 2.5</td>
<td>23.0 ± 0.9</td>
<td>35.9 ± 1.6</td>
<td>14.7 ± 1.3</td>
<td>50.6 ± 1.5</td>
</tr>
<tr>
<td>PUC20-a-E4</td>
<td>14.6</td>
<td>83.9 ± 1.8</td>
<td>47.8 ± 2.0</td>
<td>28.7 ± 1.7</td>
<td>9.0 ± 2.0</td>
<td>37.6 ± 2.1</td>
</tr>
<tr>
<td>PUC20-a-E10</td>
<td>30.0</td>
<td>74.8 ± 2.5</td>
<td>56.1 ± 1.4</td>
<td>23.0 ± 2.0</td>
<td>15.3 ± 2.1</td>
<td>38.3 ± 1.9</td>
</tr>
<tr>
<td>PUC10-r-E4</td>
<td>23.0</td>
<td>69.3 ± 1.5</td>
<td>21.7 ± 1.8</td>
<td>36.5 ± 1.6</td>
<td>13.8 ± 1.5</td>
<td>50.3 ± 1.9</td>
</tr>
<tr>
<td>PUC20-r-E4</td>
<td>14.6</td>
<td>88.3 ± 1.7</td>
<td>47.0 ± 2.5</td>
<td>30.4 ± 2.1</td>
<td>6.7 ± 2.0</td>
<td>37.1 ± 2.5</td>
</tr>
<tr>
<td>PUC20-r-E10</td>
<td>30.0</td>
<td>77.8 ± 1.1</td>
<td>56.4 ± 3.1</td>
<td>23.8 ± 3.2</td>
<td>13.4 ± 2.8</td>
<td>37.2 ± 3.0</td>
</tr>
</tbody>
</table>

$W_{PEG}$, weight percentage of PEG in sample.
REFERENCES
5. Pan JY, Li GY, Chen ZF, Chen XY, Zhu WF, Xu KT. Alternative block polyurethanes based on poly(3-hydroxybutyrate-co-4-hydroxybutyrate) and poly(ethylene glycol). Biomaterials 2009;30:2975–2984.