IUMRS-ICA 2011

Synthesis, Characterization and Evaluation of Segmented Polycaprolactone for Development of Dura Substitute

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Abstract

Molecular design and modification allow the properties of degradable materials to be suited for specific requirement of biomedical applications. For example, the dura substitute requires both mechanically and biologically suitable for repairing loss of dura mater. Here in this paper, a novel urethane linkage containing poly(ε-caprolactone) (PCL) for use as dura substitute was synthesized by solution polymerization. By using ε-caprolactone precursors with different molecular weights and ratios, a series of segmented poly(ε-caprolactone) (sPCL) were obtained via ring opening polymerization with diisocynate as coupling agent. The chemical structures of sPCL were characterized by means of GPC, IR, and 1H NMR. The effect of PCL block length on thermal behavior has been investigated using differential scanning calorimeter (DSC). Physical properties such as mechanical properties and invitro degradation rate were evaluated and the results also suggest that urethane linked PCL exhibit tunable degradation rate that is controlled by the PCL block length. sPCL membrane were fabricated and evaluated in vitro and in vivo as dura substitute.

Keywords: Poly(ε-caprolactone); dura substitute; degradation

1. Introduction

Dura mater is the outermost of three layers of meanings surrounding the brain and spinal cord, which covers and protects tissue underneath and keeps in the cerebrospinal fluid. Dural substitute are often used after a brain or neurosurgery to repair dura defects. The ideal materials for dura mater substitute, according to previous reports, must have mechanical properties such as strength and flexibility resembling healthy human dura mater and produce a watertight closure to prevent cerebrospinal leakage.
[1]. Besides, the criteria also includes biodegradable for tissue remodeling and highly biocompatible to prevent inflammation and infection. The dura substitute requires both mechanically and biologically suitable for repairing loss of dura mater.

Both synthetic polymer and naturally derived materials have been used as biomaterials for dura substitutes. Although synthetic, non-resorbable materials such as expanded polytetrafluoroethylene (ePTFE) and polyetherurethane based dura substitute are quiet popular in the market, however, such artificial dura mater remains in vivo permanently because it is non-degradable. They were also reported being chronic stimulant for surrounding tissue. These may include induction of granulation tissue formation due to foreign body reaction. The lack of contractility might cause cerebrospinal leakage as well, which led to multiple post-operation health-related concerns. Resorable, like collagenic materials though provide great clinical outcome, patients might suffers high risk of complications, allergies reaction, and infection. Alternatively, bioresorable synthetic polymers such as poly(lactide) (PLA), poly-3-hydroxybutyrate (PHB), and poly(ε-caprolactone) (PCL), are regarded to be bio-inert, non-toxic, and having tunable degradation properties [2-4]. Moreover, the required mechanical properties of polyester family above can be adjusted by copolymerization. For desired implantable applications, various modification techniques have been demonstrated to alter their characteristics. However, the lack of elasticity and unpredictable degradation period are still these biomaterials’ Achilles’ heels.

Utilizing PCL for manufacturing implant devices and drug delivery has been developed and investigated in the past decades owing to its less acidic degradation products and non-toxic nature [4-6]. In this article, we purpose a new strategy for synthesizing segmented PCLs to enable expanding their clinical applicability. sPCL differing in the segmental length of caprolactone precursors, as well as in the ratio between them have been developed and investigated. Here we demonstrated, for the first time, sPCL membrane were fabricated and evaluated in vitro and in vivo as dural substitute. The host tissue response and degradation profile of three dura substitute prototypes were characterized.

2. Experimental Preparation of Segmented Poly(ε-caprolactone) (sPCL)

2.1. Materials Synthesis

The synthesis of segmented poly(ε-caprolactone)(sPCL) was polymerized by a one shot synthesis as described below. For sPCL (PCL_{10,000}/PCL_{530,75/25}), PCL_{10000} (75g) was dissolved in 58.5g dimethylacetamide and placed in a two necked round bottomed flask. Then 14.3g H_{12}MDI and 60 mg dibutyltin dilurate (T_{12}, catalyst) were added and mixed together. After the mixture was vigorously stirred and reacted at 60 °C for a predetermined time, PCL_{530} (25g) dissolved in 58.5g dimethylacetamide were injected at 2 ml/min with a syringe to the reactor. The reaction was carried out for 8 hours under stirring and extra amount of H_{12}MDI · DMAc and T_{12} (4.3g, 117g, and 340 mg) were added into the reactor according to the viscosity of the mixture we monitored. After synthesis and purification were performed, the products were dried under vacuum for 24 hours and kept under vacuum.

2.2. Characterization

Molecular weights (Mw, Mn) and molecular weight distribution were determined by Gel Permeation Chromatography (GPC) using a Waters 515 HPLC pump and Waters 2414 RI-detector (Waters Instrument, USA) equipped with a series of columns (Shodex KF-801, KF-802, and KF-803). Fourier transform infrared spectroscopy was conducted on a Digilab FTS-3000MX FT-IR (Digilab Corp., MA, USA) spectrum analyzer to enable characterization of the common functional groups such as carbonyl groups. {\textsuperscript{1}}H and {\textsuperscript{13}}C NMR spectra were recorded with a 500 MHz Varian Unity Inova (Varian Inc., CA,
USA) high resolution NMR spectrophotometer. The composition and chemical structure of both PCL and sPCLs were therefore determined. Samples used for mechanical testing were cut into dumbbell shaped specimens for mechanical properties measurement. Tensile and elongation strength tests which follow ASTM D638 were performed using an universal material testing machine (Instron tester model 4467) equipped with a maximum 500N load cell. The tests were run at an extending speed of 150 mm/min and three specimens per sample were tested.

2.3 In-Vitro and In Vivo Degradation Studies

The degradation studies in vitro of both PCL and sPCL were performed in accordance with ISO 10993-9 standard. Samples were cut to size 2.0 x 5.0 cm with 0.5 mm in thickness. The testing specimens were dried and placed in dry box (25 °C/40% RH) for 48 hour prior study. The samples were submerged in a 50ml round bottomed flask containing 15ml phosphate buffer solution (PBS, at pH 7.4) with sealed joint. The sealed flasks were maintained at 37 °C±1 °C in a shaking incubator featuring 100 rpm shaking speed for 10 weeks. Testing pieces were removed from the solution to sample and monitor molecular weight loss by GPC weekly. The in-vivo degradation studies were carried out in the rat model of nerve injury. The tube formed samples with porosity around 80~90% were first prepared and then implanted into gap of rat sciatic nerve. Through one year period of sampling and observation of implants, the biodegradation behavior was investigated. GPC was used to determine molecular weight changes.

3. Results and Discussion

3.1 Synthesis of Segmented Poly(e-caprolactone)

Figure 1 describes the synthesis of sPCL copolymers was conducted following a ring opening polymerization and using H12MDI as a coupling agent. The strategy is based on tailor the characteristic of the copolymer by fine-tuning the composition such as molecular weight and molar ratio of PCL precursors to attain superior mechanical properties or fasten degradation period. The urethane linkages that were generated along the polymeric backbone also allowed developing strong and highly flexible biodegradable polymer.

![Polymerization of Segmented PCL polyester](image-url)

Fig. 1. Polymerization of Segmented PCL polyester.
3.2 Materials Characterization

The sPCLs were studied by NMR and FTIR. The FTIR spectra of two representative polymers, homopolymer PCL and sPCL are presented in Fig. 2. As expected, both polymers display the typical peak around 1730 cm\(^{-1}\) because of the stretch vibration of C=O in PCL. Symmetric C-H stretch vibration and asymmetric C-H stretching vibration peaks were also found at 2866 cm\(^{-1}\) and 2929 cm\(^{-1}\) in both samples, which confirms the successful synthesis of PCL and sPCL containing signature caprolactone sequence. In addition, sPCL spectra displays the peak of amide (NH) stretching absorption around 3363 cm\(^{-1}\) and hydrogen bonded NH stretch absorption around 1550 cm\(^{-1}\). These results reveal that the urethane linked segmented PCL were successfully synthesized.

![FTIR spectra of PCL homopolymer and modified PCL.](image)

Mechanical properties of segmented PCL were measured and compared to those of homogeneous PCL dumbbell test pieces using the same fabrication method. The tensile strength of these polymers presented in Fig. 3. Noticeably, while PCL homopolymer and sPCL (PCL\(_{530}/PCL\(_{10000}\) ratio 25/75, crystallinity 44.8%) display similar crystallinity, the sPCL exhibited remarkably high tensile strength up to 36 MPa, whereas homopolymer only reaches approximately less than half of the value, 16 MPa. This may be due to the urethane linkages in polymer chains and therefore forming strong hydrogen bonds. These inter-molecular hydrogen bonds increase the force among polymer chain, as the results, the tensile strength were doubled up.

![The tensile properties of PCL and segmented PCLs.](image)
3.3 In vivo Studies

The porous PLA and sPCL (area porosity 73%) membranes were implanted into mice for a month to investigate the biocompatibility. As shown in Fig. 4, histological examination results of the sPCL film elicit a minimal initial tissue reaction, which is transient and is followed by the deposition of fibrous, connective layers of tissue. Figure 5 also demonstrate that after 3 months post implantation in rabbit, the sPCL dura substitute prototype their porous structure allows for the progressive integration of the implant by the newly formed fibrous, connective tissue with minimal risk of CSF leakage and inflammation, while foreign body reaction was found in commercial available product. There were also no tissue adhesion of sPCL on the pia mater was observed.

![Histological examination result of sPCL](image)

Fig. 4. HS staining result shows great biocompatibility of sPCL.

![In vivo study of Dura Mater substitution in rabbits](image)

Fig. 5. In vivo study of Dura Mater substitution in rabbits(from left to right: sPCL membrane, PLGA and commercial product).

4. Conclusions

A new strategy for synthesizing novel urethane linked biodegradable poly-caprolactone with controlled mechanical properties and fasten hydrolytic degradation process comparable to PCL is reported. The segmented polycaprolactone with urethane linkage introducing into polymer chain exhibited high flexibility and remarkable tensile strength above 200%. Our in vivo results also reveal that urethane linked polycaprolactone is highly biocompatible and could serve as dura substitute. The implant may elicit a minimal initial tissue reaction, which is transient and is followed by the deposition of fibrous, connective layers of tissue. Long term follow-up and a large number of patients are still necessary to confirm the absence of complication related to sPCL Dural prototype. More tissue engineering application awaits us to explore.
Acknowledgements

The authors would like to thank Industrial Technology Research Institute, Taiwan, ROC and Ministry of Economic Affairs, Taiwan, ROC for financial support.

References