Poly (Terephthaloyl Diglyceride-co-1, 2-cyclohexanediacyl Diglyceride) - A Biodegradable and Biocompatible Functional Polymer for Biomedical Applications

Yangfen Xie\textsuperscript{1}, Lijie Sun\textsuperscript{2}, Xiaoping Bi\textsuperscript{3,4}, Yifan Guo\textsuperscript{2}, Shuo Chen\textsuperscript{2}, Xianqun Fan\textsuperscript{3,4}, Fengling Qing\textsuperscript{1} and Zhengwei You\textsuperscript{2,*}

\textsuperscript{1} State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, 2999 North Renmin Road, Shanghai 201620, China
\textsuperscript{2} State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Materials Science and Engineering, Donghua University, 2999 North Renmin Road, Shanghai 201620, China
\textsuperscript{3} Department of Ophthalmology, Ninth People's Hospital, Shanghai JiaoTong University School of Medicine, No.639 Zhizaoju Road, Shanghai, 200011, China.
\textsuperscript{4} Shanghai Key Laboratory of Orbital Diseases and Ocular Oncology, Shanghai, 200011, China.

*Corresponding author: zyou@dhu.edu.cn; Tel: (+86 02167874279)

Abstract. Biodegradable polyesters have been widely used in biomedical engineering. Introduction of functional groups to polyesters will expand their applications. Herein, we developed a functionalizable copolyester with free hydroxyl groups (-OH) - poly (terephthaloyl diglyceride-co-1,2-cyclohexanediacyl diglyceride) (PTeCD). PTeCD was readily synthesized by acid-induced epoxide ring-opening polymerization in one step. Its structure was characterized by NMR and FTIR. PTeCD exhibited a good cytocompatibility and biodegradability. We expect PTeCD will be a useful polymer for biomedical applications.

Key words: biodegradable, aliphatic-aromatic copolyester, functional

1. Introduction
Biodegradable polymers have been used in a wide range of emerging biomedical applications including temporary prostheses, tissue engineering scaffolds, and drug delivery vehicles [1]. Aliphatic polyesters such as poly(ε-caprolactone) (PCL) and poly(lactic acid) (PLA) represent one of the most important classes of biodegradable polymeric biomaterials [1-3]. Comparing with enzymatically degradable polymers, aliphatic polyesters are usually hydrolysable, and thus, are preferred for in vivo applications due to the minimal site-to-site and patient-to-patient variation of hydrolysis [4]. To meet the diverse requirements of biomedical applications, the properties of polymeric biomaterials are expected to be modulated in a wide range. Introduction of aromatic moieties to the polymer structure is an efficient way to modulate the properties such as thermal and mechanical performance [5]. However, compared to aliphatic polyesters, aromatic polyesters have been less studied as biodegradable biomaterials partly due to their limited biodegradability. Furthermore, most synthetic polymeric biomaterials including polyesters are generally biologically inert and lack of reactive groups...
for modifications, which limit the modulation of their properties and consequent applications [6-7]. Introduction of functional groups could be one way to address aforementioned drawbacks. Functional groups such as hydroxyls can directly tune the physical, chemical and biological properties of polymers. Furthermore, modification of these functional groups provides access to diverse polymers with a wide range of properties [4, 8-9]. However, chemical synthesis of functional polyesters is still a challenge in polymer science, because it usually involves a complex multistep process with a low overall yield [10-12]. Recently, we established a new simple and versatile method, acid-induced epoxide ring-opening polymerization, which could efficiently synthesize polyesters with pendent hydroxyl groups from a variety of diacids and diepoxides in one step [13]. It is applicable on both aromatic and aliphatic substrates [14-15]. Accordingly we set out to use this new developed method to synthesize functional aliphatic/aromatic copolyesters, which would be useful biodegradable polymers with tunable properties. Here, we report one example, poly(terephthaloyl diglyceride-co-1,2-cyclohexanediacetyl diglyceride) (PTeCD) (Figure 1). We designed this functional polyester according to following consideration. PTeCD contains alternating aliphatic and aromatic repeating units. PTeCD are expected to be biodegradable. In addition, PTeCD has extensive hydroxyl groups, which would improve the hydrophobic nature of aromatic structure and make PTeCD to have suitable hydrophilicity for biomedical applications. More importantly, hydroxyl groups are robust for transformations including facile biofunctionalization with various biomolecules such as peptide and gene. Thus, PTeCD would be a versatile intermediate for a series of functionalized biomaterials.

2. Experimental Section

2.1 Materials and reagents
Diglycidyl 1,2-cyclohexanedicarboxylate (J&K, 95%), terephthalic acid (Acros Organics, 98%), tetrabutylammonium bromide (Acros, ≥99%), anhydrous solvents N,N-dimethylformamide (DMF), and 1,6-hexylidisocyanate (Sigma, ≥99%) were used without further purification. Deuterated dimethyl sulfoxide (DMSO-d<sub>6</sub>) for NMR analysis was purchased from Cambridge Isotope Laboratories, Inc. All other solvents were purchased from Sinopharm Chemical Reagent Co., Ltd.

2.2 Synthesis and characterization of PTeCD
Diglycidyl 1,2-cyclohexanedicarboxylate, an equal molar amount of terephthalic acid, and 0.6 mol% tetrabutylammonium bromide were mixed and dissolved in anhydrous DMF into a three-neck round-bottom flask in a glove box filled with nitrogen. The flask was transferred out of the glove box in a sealed state, and connected to nitrogen. Then, the contents in flask were stirred under a nitrogen atmosphere at 100 °C for 26 h. The reaction mixture was purified via precipitation in ethyl ether for three times, and vacuum-dried at ambient temperature for 24 h to yield PTeCD.

The molecular weight and distribution of PTeCD were determined via Waters gel permeation chromatography (GPC) using a differential refractive index detector and a Brookhaven multi-angle light scattering detector. The measurement was performed at 40 °C using DMF (HPLC grade) as the eluent. 1H nuclear magnetic resonance (NMR) spectrum was obtained in DMSO-d<sub>6</sub> using a Bruker 400 NMR (400 MHz) spectrometer at 25 °C. Fourier transformed infrared (FTIR) was carried out using a Thermo Nicolet 6700 spectrometer with a total of 32 scans from 500 to 4000 cm<sup>-1</sup>.

2.3 Evaluation of in vitro biocompatibility of PTeCD
The wells of a tissue culture-treated polystyrene (TCPS) 24-well plate were coated with 80 μl of 1 g/l 2,2,2-trifluoroethanol solution of PTeCD that filtered through a 0.2 μm sterilization filter. The plate was dried under vacuum overnight to evaporate the residual solvent in polymer coating. Both the PTeCD-coated plate and TCPS plate were sterilized under UV light for 30 min, then washed with 1 ml phosphate buffered saline (Lonza) at least three times and with 1 ml culture medium once with gentle shaking (20 rpm). Each well was seeded with 50,000 nonimmortalized rat primary osteoblasts obtained from neonatal (2–3 days old) Spratue-Dawley rats, and isolated by sequential trypsin-collagenase digestion of calvaria. The cells were cultured in Dulbecco’s phosphate-buffered saline (DPBS) supplemented with Dulbecco’s Modified Eagle Medium (DMEM) and fetal calf serum at 37 °C with 5% CO2. The culture medium was exchanged every 2 days. The cell adhesion rate was used as a quantitative evaluation of osteoblast adhesion onto PTeCD coating and TCPS plates (n = 5). Osteoblasts were cultured for 3 and 24 h on the polymer-coated plates. At each time point, the adhered osteoblasts were enzymatically (0.25% trypsin–0.1% EDTA; Gibco) detached and counted using hemocytometer. The cell adhesion rate was expressed as a percentage of the initial number of seeded cells (2 × 10^4 cells per well).

2.4 In vitro degradation of PTeCD
Disk-shaped sample of PTeCD (n = 4) with dimensions of 5 mm × 2 mm (diameter × thickness) was evenly cut into 4 pieces of fan-shaped specimens. Every specimen was weighted and incubated at 37 °C in 1 ml DPBS solution with a pH of 7.2-7.4. At the predetermined period of times, buffer solution was exchanged, and the samples washed with deionized water and lyophilized at least 24 h in sequence. Mass remained of sample was determined by the following formula (where M0 and Mt are the initial mass and dry-weight at each time point, respectively):

\[
\text{Mass remained} \, (\%) = \frac{M_t}{M_0} \times 100 \, \%
\]  

3. Results and Discussion

3.1 Polymer synthesis and characterization

\[ \text{Figure 2. Synthesis route of PTeCD.} \]

PTeCD (Mn = 9.2 kD, PDI = 2.1) was prepared by acid-induced epoxide ring-opening polymerization between commercially available diglycidyl 1,2-cyclohexanedicarboxylate and terephthalatic acid in the presence of a catalytic amount of tetrabutylammonium bromide (Bu4NB).
The structure of PTeCD was characterized by $^1$H NMR and FTIR. In the $^1$H NMR spectrum (Figure 3A), the signal marked ‘a’ at chemical shift δ values of 8.06 ppm was assigned to the CH protons of benzene ring. The three signals marked ‘c, d and e’ at chemical shift δ values of 2.88, 1.69-1.85 and 1.33 ppm were ascribed to the three different protons of the cyclohexane, respectively. The signals of CH and CH$_2$ protons in the glyceryl moiety marked ‘b’ appeared at δ 3.19-5.45 ppm. To further confirm the presence of the characteristic functional groups in PTeCD, FTIR spectrum was recorded. As shown in Figure 3B, a broad and intense peak at ~3489 cm$^{-1}$ was in accordance with the stretching vibration of the O-H bonds. The intense peak around 1731 cm$^{-1}$ was assigned to C=O stretching vibration, confirming the formation of ester bonds. Skeletal vibration of benzene ring displayed signals at 1506 cm$^{-1}$ and 1577 cm$^{-1}$, as well as the C-O and C-H stretching vibration at 1278 cm$^{-1}$ and 2947 cm$^{-1}$, respectively.

3.2 Evaluation of in vitro biocompatibility of PTeCD.

The in vitro biocompatibility of PTeCD was evaluated by culturing rat primary osteoblasts on PTeCD-coated 24-well plates using pristine TCPS as a control. The osteoblasts cells showed similar polygonal morphology on PTeCD (Figures 4A and 4B) and TCPS (Figures 4C and 4D). Moreover, the adhesion of osteoblasts on PTeCD was investigated by quantitative counting adhered cells on the
surface at 3 and 24 hours after seeding. As shown in Figure 4E, the cell adhesion rate on PTeCD coating was at a similar level to that on TCPS at each time point, reaching 66.8 ± 7.0% and 82.8 ± 11.9% at 3 and 24 h, respectively. This indicated that PTeCD supported osteoblast adhesion. These results reveal PTeCD had a good cytocompatibility.

3.3. In vitro degradation of PTeCD.

![Figure 5. In vitro degradation of PTeCD in DPBS.](image)

We evaluated the in vitro degradation of PTeCD in DPBS. PTeCD showed a steady degradation with a remained mass of 92.5 ± 2.0% at day 28 (Figure 5). This confirmed the degradability of PTeCD.

4. Conclusions

An aliphatic-aromatic copolyester (PTeCD) with free hydroxyl groups was designed and successfully synthesized in one step from commercially available materials. PTeCD exhibited good cytocompatibility and degradability. The modification of free hydroxyl groups of PTeCD can modulate its physical, mechanical, and biological properties. We expect PTeCD will be a useful polymer for biomedical applications.

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References

[1] L. Wang, S. Venkatraman and L. Kleiner, *Journal of controlled release : official journal of the Controlled Release Society*, 2004, **99**, 207-216.
[2] J. W. Leenslag and A. J. Pennings, *Macromol. Chem. Phys.*, 1987, **188**, 1809-1814.
[3] D. Garlotta, *J. Polym. Environ.*, 2001, **2**, 63-84.
[4] M. Vert, *Biomacromolecules*, 2005, **6**, 538-546.
[5] I. Pomerantseva, N. Krebs, A. Hart, C. M. Neville, A. Y. Huang and C. A. Sundback, *J Biomed Mater Res A*, 2009, **91**, 1038-1047.
[6] L. S. Nair and C. T. Laurencin, *Prog. Polym. Sci.*, 2007, **32**, 762-798.
[7] Z. Ma, Z. Mao and C. Gao, *Colloids Surf B Biointerfaces*, 2007, **60**, 137-157.
[8] D. S. Benoit, M. P. Schwartz, A. R. Durney and K. S. Anseth, Nat. Mater., 2008, 7, 816-823.
[9] C. K. Williams, Chem. Soc. Rev., 2007, 36, 1573-1580.
[10] D. E. Noga, T. A. Petrie, A. Kumar, M. Weck, A. J. García and D. M. Collard, Biomacromolecules, 2008, 9, 2056-2062.
[11] R. J. Pounder and A. P. Dove, Polym. Chem., 2010, 1, 260-271.
[12] S. Ponsart, J. Coudane and M. Vert, Biomacromolecules, 2000, 1, 275-281.
[13] Z. You, H. Cao, J. Gao, P. H. Shin, B. W. Day and Y. Wang, Biomaterials, 2010, 31, 3129-3138.
[14] Z. W. You, X. P. Bi and Y. D. Wang, Macromol. Biosci., 2012, 12, 822-829.
[15] Z. You and Y. Wang, Adv. Funct. Mater., 2012, 22, 2812-2820.