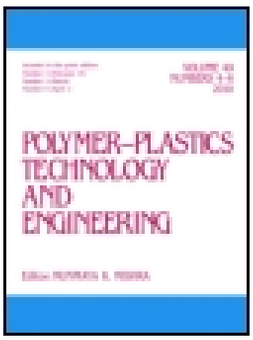


Taylor & Francis
Taylor & Francis Group

Polymer-Plastics Technology and Engineering

ISSN: 0360-2559 (Print) 1525-6111 (Online) Journal homepage: http://www.tandfonline.com/loi/lpte20

Bulk Synthesis of Monodisperse and Highly Biocompatible Poly (ε-caprolactone)-diol by Transesterification Side-Reactions

Mohsen Shahrousvand, Gity Mir Mohamad Sadeghi, Ali Salimi & Mohammad Nourany

To cite this article: Mohsen Shahrousvand, Gity Mir Mohamad Sadeghi, Ali Salimi & Mohammad Nourany (2016): Bulk Synthesis of Monodisperse and Highly Biocompatible Poly (ε-caprolactone)-diol by Transesterification Side-Reactions, Polymer-Plastics Technology and Engineering, DOI: 10.1080/03602559.2016.1211690

To link to this article: http://dx.doi.org/10.1080/03602559.2016.1211690

Accepted author version posted online: 16 Sep 2016.
Published online: 16 Sep 2016.

Bulk Synthesis of Monodisperse and Highly Biocompatible Poly (ε-caprolactone)-diol by Transesterification Side-Reactions

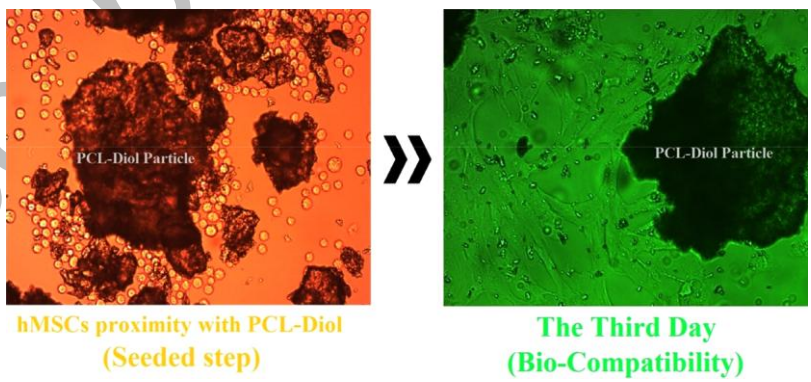
Mohsen Shahrousvand[1], Gity Mir Mohamad Sadeghi[1], Ali Salimi[2], Mohammad Nourany[1]

[1]Department of Polymer Engineering and Color Technology, Amirkabir University of Technology, Tehran, Iran
[2]Nanobiotechnology Research Center, Baqiyatallah University of Medical Science, Tehran, Iran

Abstract

Ring opening polymerization of ε-caprolactone was carried out at 130 °C, under partial vacuum in the presence of stannous octoate as the catalyst and 1, 4-Butanediol as the initiator. After the termination of polymerization by deionized water, a hydroxyl group formed at the end of the polymer chains. Structure of the synthetic poly (ε-caprolactone)-Diols (PCL-Diol), molecular weight, polydispersity index (PDI) and Cell viability evaluated. Very narrow distribution in the molecular weight obtained for PCL-Diols is due to a new method for synthesis. It was shown that by the increase of PCL-Diols, the compatibility of human mesenchymal stem cells grew up.

GRAPHICAL ABSTRACT



KEYWORDS: Polycaprolactone Diol, Biocompatible, Transesterification, MTT assay

1. INTRODUCTION

Poly (ε-caprolactone) (PCL) is a biodegradable and bioresorbable polyester based on lactone monomers which is a hydrophobic and semi-Crystalline polymer [1, 2]. However, the crystallinity degree of the polymer decreases as molecular weight increases [3, 4]. Since biocompatibility of polymers is defined by an appropriate response to a particular application in the host environment, PCL is a suitable choice [5-8]. Although initially the use of PCL was overwhelmed by the popularity of other biodegradable polymers due to poor mechanical properties in some medical applications that bearing a high load was a prerequisite. But in the recent years with progress in tissue engineering, PCL was considered more useful due to its feasible biological, rheological and viscoelastic properties [9]. PCL, other than being used as homopolymer in scaffolds fabrication [10], is also used as blends [11-16], composites [17-21] and copolymers [22] with other natural and synthetic polymers for drug delivery systems [23-25], scaffolding [6, 10, 19, 20, 26] and shape memory applications [27-30].

There are two general methods for the synthesis of PCL: Polycondensation of 6-hydroxycaproic (6-Hydroxyhexanoic) acid monomer and ring opening polymerization (ROP) of ε-caprolactone. In the first method, in order to extract the produced water molecules from condensation medium, the reaction is carried out under a vacuum and usually without any catalysts and other additives. In the second method, there are two mechanisms: general mechanisms and transesterification side-reactions. General mechanisms can be divided into four categories: a. Anionic ROP, b. Cationic ROP, c. Monomer-activated ROP, d. Coordination–insertion ROP. In transesterification side-

reactions mechanism, lactone monomer is polymerized by using an initiator and a catalyst [31, 32]. One of the catalysts that is used extensively in the current mechanism is stannous octoate (SO) due to low cost, low toxicity and high performance [3, 33, 34].

The present paper aims to report bulk polymerization of ε-caprolactone in the presence of SO as a catalyst and butanediol (BDO) as initiator carried out at 130°C in order to synthesize monodisperse and highly biocompatible poly (ε-caprolactone)-diol. Chemical, thermal, mechanical properties and toxicity studies of the synthesized polymers are evaluated. Hydroxyl groups were created at the ends of PCL chains to be used as macrodiol in the synthesis of polyurethane for tissue engineering applications [35-39]. Unless to use the synthesized PCl-Diol for preparation of polyurethane based scaffolds is a narrow molecular weight distribution. In this new method for synthesis, we could prepare more monodisperse PCL-Diols than commercial types.

2. MATERIALS AND METHODS

2.1. Materials

ε-Caprolactone (CL) and 1,4-Butanediol (BDO) were purchased from Acros and Sigma-Aldrich respectively. Tin (II) 2-ethylhexanoate (or stannous octoate) (SO) which is used as the initiator of this study was purchased from Sigma-Aldrich. Monomer, initiator and catalyst were dehydrated by momentum vacuum pump setup[40, 41] and stored under vacuum until used. Mesenchymal Stem Cells (hMSCs) were obtained from Stem Cell Technology Research Center and maintained in a T-75 culture flask. Cells were maintained in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham

(DMEM/F-12 Ham) supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin and streptomycin. MTT (3-(4, 5-dimethyl-thiazol- 2-yl)-2, 5-diphenyl tetrazolium bromide) was purchased from Sigma. Double-distilled deionized water was used at various stages of the work.

2.2. Bulk Polymerization Of E-Caprolactone

According to Table.1 specified amounts of CL and BDO were added to the glass reactor from their containers which were under partial vacuum. First, we poured BDO and SO into the reactor and then put the mixture under nitrogen purge and vacuum for three continuous rounds. After keeping the reactor's temperature at 80 °C for 10 minutes, the temperature was increased to 130°C gradually whilst adding the monomers. In order to terminate the reaction two hours after adding the last drop of monomer, water was added to the reactor. The contents of the reactor were transferred to a vial with a rubber lid and placed in vacuum oven to remove excess water. These polymers would be used for subsequent works.

2.3. MTT Assay And Cell Viability

Due to the use of PCL-diols for the differentiation of human Mesenchymal Stem Cells (h-MSCs) to osteoblasts for bone tissue engineering purposes for the further parts of this study, h-MSCs were selected to evaluate cytotoxicity. All experiments were ran three times. PCL-diols were used in four different doses in order to evaluate the effect of polymer on cell proliferation on cell culture in 48-well plates: 0.0005, 0.0015, 0.0025 (g per well) (PCL-diol particles) and 0.1g coated film. The samples were sterilized using

ethanol (70%w/w) and UV radiation. hMSCs were cultured into 48-well plates which were loaded by PCL-diols at a density of 10,000 cells/well and then transferred into an incubator. MTT assay was provided for the first, third and fifth days. For each assessment, the culture solution in wells was removed and washed with DMEM (without FBS). The MTT solution was added to the wells and incubated for three hours. After removing MTT solution and washing wells with DMEM, 100 microliter of DMSO (while the oven lamp was turned off) was added to each well and pipetted several times. The contents of each well was transferred to a 96-well plate and the optical density (OD) was measured by ELISA plate reader (540-630nm). The relative cell viability (%) was then compared to control cells containing cell culture medium without polymer and then calculated by using the Eq. (1) ($OD^{'}$ = the absorbance value of experimental wells minus zero wells; avg($ODC^{'}$) = the average absorbance value of corrected control wells):

$$\text{Relative cell viability } \% = \frac{OD^{'}}{\text{avg } ODC^{'}} \times 100 \qquad (1)$$

2.4. Characterization

Fourier transform infrared (FT-IR) analysis for synthesized PCL-diols was conducted with a Thermo Nicolet 670 spectrometer from 4000 to 400 cm^{-1} at a spectral resolution of 4 cm^{-1} using KBr method. The molecular weight of the polymers and their polydispersity index (PDI) was measured by gel-permeation chromatography (GPC 2000, WATERS USA) (polymers solved in THF). Melting and crystallization characteristics of PCL-diols were examined by using a Mettler/Toledo DSC system. Samples with 8–9 mg weight were encapsulated in aluminum pans and treated at heating/cooling ramps with the rate of

10°C/min. The applied atmosphere was nitrogen with a flow rate of 45 mL/min. To evaluate the crystallization behavior of PCL-diols without the interference of thermal history, after the first run of heating, the cooling ramp was set to -57°C and, subsequently, the second ramp of heating was used. The crystallinity of PCL calculated from Eq. (2):

$$X_c = \Delta H_f - \Delta H_c \ / \Delta H_f^0 \times 100 \qquad (2)$$

Where X_c is percent crystallinity, ΔH_f and ΔH_c are the heat of fusion and crystallization of PCL-diols, respectively and ΔH^0_f (= 136 J/g) is the heat of fusion of 100% crystalline PCL-diol [42].

Tensile properties were evaluated on an Instron tensile testing apparatus (5566-Applied Science Co., Ithaca, NY). Tensile strength and elongation were measured at a crosshead speed of 1 mm/min. During the Evaluation of cell viability in different days, cells were photographed by a Leica Leitz light Microscope (Leica Inc., Foster City, CA) and scanning electron microscopy (Philips XL30 SEM and gold sputter coating instrument). The amount of formazan salt in MTT assay was determined by measuring the OD at 540-630 nm using a microplate reader (Tecan Austria GmbH, Austria). Relative cell viability was determined by the amount of MTT converted into formazan salt. PCL-diols were marked as PCL-diol-x, where x is the molecular weight of PCL-diols.

3. RESULTS AND DISCUSSION

3.1. Structural And Thermal Studies

Poly (ε-caprolactone)-diol (PCL-diol) was synthesized from ε-caprolactone and butanediol (BDO) as initiator in transesterification side-reactions polymerization process in the presence of Tin (II) 2-ethylhexanoate (or stannous octoate) (SO) as a catalyst according to Scheme 1. Tin (II) 4-hydroxybutan-1-olate in step 1 was obtained by the reaction of SO and BDO to attack the CL rings and stannous poly alkoxide complex (I) was formed (Scheme 1.B). If in stannous poly alkoxide complex (I) n equals 2m, final PCL-diols will be monodispersed. This complex can react with the hydroxyl groups present in the water or BDO. If this complex reacts with water molecules, polymerization will be terminated (Scheme 1.C). If stannous poly alkoxide complex (I) reacts with BDO, one termination reaction and one initiation reaction will occur simultaneously (Scheme 1.D). Therefore, the presence of water and BDO during the polymerization reactions will cause the increase of polydispersity in PCL-diols molecular weight.

Polymerization was carried out according to what is mentioned in Table 1. FT-IR spectra of PCL-diols are shown in Figure 1. According to Table 2, the band at 1294 cm^{-1} is assigned to the backbone C–C and C–O stretching modes in the crystalline PCL-diol. PCL-diols display three characteristic peaks at 1727, 1293, and 1240 cm^{-1} (C[dbond]O and –COO– stretching) as well as 3510 cm^{-1} (OH stretching). In comparison of PCL-diols, whenever molecular weight is reduced, the total amount of hydroxyl groups increases by the same polymer mass.

Molecular weights of the PCL-diols were measured by GPC and are shown in Table 3. By the proposed mechanism the difference between theoretical and experimental

molecular weight is negligible. According to the data, it seems that the absence of water during the polymerization reactions affected monodispersity.

The molecular weight distribution (PDI) becomes slightly wider by increasing molecular weight due to increase of entanglement and reduction in the rate of monomer penetration. Figure 2 show crystallization and melting curves of PCL-diols. The characteristics of these curves, including melting and crystallization temperatures (T_m and T_c), melting and crystallization enthalpies (ΔH_m and ΔH_c), and the onset temperature of crystallization (Tcs) have been gathered in Table 3. The crystallinity of PCL-diols increased by increasing molecular weight and then dropped. This phenomenon is due to the critical molecular weight at which the entanglement occurs[46].

3.2. Mechanical Tests

The characteristic runs from the tensile testing of PCL-diols are shown in Fig. 3. According to the graph, all three samples showed brittle behavior. Although the mechanical behavior of three samples is almost the same, but PCL-diol-8300 showed higher strength due to further crystallinity. PCL-diol-16200 broke in higher strain in comparison to other samples due to the presence of more amorphous regions and entanglement. Differences in the mechanical behavior of samples depend on the amount of crystallinity and molecular weight[47]. Modulus of PCL-diol-3500, PCL-diol-8300 and PCL-diol-16200 are 21.36, 27.66 and 12.19 MPa respectively. Elongation at break for PCL-Diol-16200 occurred at 4%.

3.3. Biocompatibility Evaluation

PCL is a biocompatible polymer[48] which is in accordance to Figure 4 (a and b). On the first day there is a huge growth for the cells for all three samples. In each day by increasing the amount of polymer in the cellular environment, cell growth increased. Fig 4 (b) and Fig 5 clearly show that the cells have a higher accumulation around polymer particles.

The graph shows that the proliferation of stem cells was certainly not tumor-like, because the growth rate in the third and fifth day was approximately constant. This is simply specified in the relative cell viability charts (Fig 6). It is clear that the proliferation for cells has greatly increased in the first day compared to control sample. Following an increase in polymer dosage, cell viability increased relatively and PCL-diols showed an encouraging role in the proliferation and no toxicity was observed. Fig. 6 (*a*-*c*) determines that there is a persuasiveness behavior only on the first day and wells in 48-well plate are almost covered completely by cells. Optical microscope images confirm that polymer containing wells provide a high proliferation rate for cells on the first day of experiment (Fig 7).

4. CONCLUSIONS

PCL-diols with different molar ratio and molar mass were synthesized by transesterification side-reactions polymerization process using stannous octoate (SO) as catalyst and butanediol (BDO) as initiator under relative vacuum by momentum vacuum pump setup. Three types of molecular weight of PCL-diols were synthesized by changing

the molar ratio of monomer, initiator and catalyst molecules as 3500, 8300 and 16200 g/mol. According to the proposed mechanism, the absence of water in the first two stages (initiation and propagation) of synthesis leads to monodisperse molecular weight distribution. Molecular structures, thermal properties, biocompatibility and the mechanical properties of PCL-diols changed considerably when molecular weight changed. The more molecular weight increased, the more crystallinity increased, until it reached the entanglement molecular weight. Then crystallinity decreased by increasing molecular weight. Highest degree of crystallinity was related to PCL-diol-8300 with 20.35%. Crystallization and melting temperatures for all samples were about 30°C and 55°C respectively. The mechanical behavior of PCL-diols was similar and all were brittle. The highest elongation at break was related to PCL-diol-16200 with 4% and the highest modulus was related to PCL-diol-8300 with 27.66 Mpa. PCL-diols showed high biocompatibility in vicinity of hMSCs. All samples had a higher relative cell viability compared to control groups for all days. In wells of 48-well plate, as the amount of PCL-diols increases, the growth of cells increases.

ACKNOWLEDGMENTS

Authors thank Amirkabir University of Technology (Tehran Polytechnic) for general university funding and Mrs. Karimi and Mrs. Ghadamali for support in the sample preparation for GPC and FTIR.

COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST

Financial support from Amirkabir University of Technology (Tehran Polytechnic) is gratefully acknowledged.

REFERENCES

[1] Iqbal M, Valour J-P, Fessi H, Elaissari A. Preparation of biodegradable PCL particles via double emulsion evaporation method using ultrasound technique. Colloid and Polymer Science 2015;293:861-73.

[2] Su S-K, Wu C-S, Siao J-W, Yen F-S, Wu J-Y, Huang CM. Biodegradable blends prepared from polycaprolactone and poly (glutamic acid): Structure, thermal properties, and biodegradability. Polymer-Plastics Technology and Engineering 2010;49:1361-70.

[3] Storey RF, Sherman JW. Kinetics and mechanism of the stannous octoate-catalyzed bulk polymerization of ε-caprolactone. Macromolecules 2002;35:1504-12.

[4] Nair LS, Laurencin CT. Biodegradable polymers as biomaterials. Progress in polymer science 2007;32:762-98.

[5] Pitt CG, Chasalow F, Hibionada Y, Klimas D, Schindler A. Aliphatic polyesters. I. The degradation of poly (ϵ-caprolactone) in vivo. Journal of Applied Polymer Science 1981;26:3779-87.

[6] Hutmacher DW, Schantz T, Zein I, Ng KW, Teoh SH, Tan KC. Mechanical properties and cell cultural response of polycaprolactone scaffolds designed and fabricated via fused deposition modeling. Journal of biomedical materials research 2001;55:203-16.

[7] Williams D. Revisiting the definition of biocompatibility. Medical device technology 2003;14:10-3.

[8] Williams DF. On the mechanisms of biocompatibility. Biomaterials 2008;29:2941-53.

[9] Woodruff MA, Hutmacher DW. The return of a forgotten polymer—polycaprolactone in the 21st century. Progress in Polymer Science 2010;35:1217-56.

[10] Zander NE, Orlicki JA, Rawlett AM, Beebe Jr TP. Electrospun polycaprolactone scaffolds with tailored porosity using two approaches for enhanced cellular infiltration. Journal of Materials Science: Materials in Medicine 2013;24:179-87.

[11] Sarasam A, Madihally SV. Characterization of chitosan–polycaprolactone blends for tissue engineering applications. Biomaterials 2005;26:5500-8.

[12] Sarasam AR, Krishnaswamy RK, Madihally SV. Blending chitosan with polycaprolactone: effects on physicochemical and antibacterial properties. Biomacromolecules 2006;7:1131-8.

[13] Semba T, Kitagawa K, Ishiaku US, Hamada H. The effect of crosslinking on the mechanical properties of polylactic acid/polycaprolactone blends. Journal of applied polymer science 2006;101:1816-25.

[14] Lim J, Chong MSK, Teo EY, Chen GQ, Chan JK, Teoh SH. Biocompatibility studies and characterization of poly (3- hydroxybutyrate- co- 3- hydroxyhexanoate)/polycaprolactone blends. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2013;101:752-61.

[15] Shadi L, Karimi M, Entezami AA. Preparation of electroactive nanofibers of star-shaped polycaprolactone/polyaniline blends. Colloid and Polymer Science 2015;293:481-91.

[16] Kumar S, Varma IK. Degradation Studies of Thermoplastics Composites of Jute Fiber–Reinforced LDPE/Polycaprolactone Blends. Polymer-Plastics Technology and Engineering 2006;45:1219-25.

[17] Sayyar S, Murray E, Thompson BC, Gambhir S, Officer DL, Wallace GG. Covalently linked biocompatible graphene/polycaprolactone composites for tissue engineering. Carbon 2013;52:296-304.

[18] Hassan MI, Sun T, Sultana N. Fabrication of nanohydroxyapatite/poly (caprolactone) composite microfibers using electrospinning technique for tissue engineering applications. Journal of Nanomaterials 2014;2014.

[19] Ghavimi SAA, Ebrahimzadeh MH, Shokrgozar MA, Solati-Hashjin M, Osman NAA. Effect of starch content on the biodegradation of polycaprolactone/starch composite for fabricating in situ pore-forming scaffolds. Polymer Testing 2015;43:94-102.

[20] Won J-E, Mateos-Timoneda MA, Castano O, Planell JA, Seo S-J, Lee E-J, et al. Fibronectin immobilization on to robotic-dispensed nanobioactive glass/polycaprolactone scaffolds for bone tissue engineering. Biotechnology letters 2015;37:935-42.

[21] Abdolrasouli MH, Sadeghi GMM, Nazockdast H, Babaei A. Polylactide/Polyethylene/Organoclay Blend Nanocomposites: Structure, Mechanical and Thermal Properties. Polymer-Plastics Technology and Engineering 2014;53:1417-24.

[22] Yu H, Wang W, Chen X, Deng C, Jing X. Synthesis and characterization of the biodegradable polycaprolactone- graft- chitosan amphiphilic copolymers. Biopolymers 2006;83:233-42.

[23] Coombes A, Rizzi S, Williamson M, Barralet J, Downes S, Wallace W. Precipitation casting of polycaprolactone for applications in tissue engineering and drug delivery. Biomaterials 2004;25:315-25.

[24] Dash TK, Konkimalla VB. Poly-ϵ-caprolactone based formulations for drug delivery and tissue engineering: A review. Journal of Controlled Release 2012;158:15-33.

[25] Mishra GP, Tamboli V, Mitra AK. Effect of hydrophobic and hydrophilic additives on sol–gel transition and release behavior of timolol maleate from polycaprolactone-based hydrogel. Colloid and polymer science 2011;289:1553-62.

[26] Díaz E, Puerto I, Sandonis I, Ibañez I. Morphology and mechanical properties of PLLA and PCL scaffolds. Polymer-Plastics Technology and Engineering 2014;53:150-5.

[27] Zhang F, Zhou T, Liu Y, Leng J. Microwave synthesis and actuation of shape memory polycaprolactone foams with high speed. Scientific reports 2015;5.

[28] Lendlein A, Langer R. Biodegradable, elastic shape-memory polymers for potential biomedical applications. Science 2002;296:1673-6.

[30] Quadrini F, Bellisario D, Santo L, Gaudio CD, Bianco A. Shape memory foams of microbial polyester for biomedical applications. Polymer-Plastics Technology and Engineering 2013;52:599-602.

[31] Labet M, Thielemans W. Synthesis of polycaprolactone: a review. Chemical Society Reviews 2009;38:3484-504.

[32] Deanin RD. New useful polymers by ring-opening polymerization. Polymer-Plastics Technology and Engineering 1992;31:229-39.

[33] Wang L, Poirier V, Ghiotto F, Bochmann M, Cannon RD, Carpentier J-Fo, et al. Kinetic Analysis of the Immortal Ring-Opening Polymerization of Cyclic Esters: A Case Study with Tin (II) Catalysts. Macromolecules 2014;47:2574-84.

[34] Albertsson A-C, Varma IK. Recent developments in ring opening polymerization of lactones for biomedical applications. Biomacromolecules 2003;4:1466-86.

[35] Moore TG, Adhikari R, Gunatillake PA. Biodegradable polyurethane and polyurethane ureas. Google Patents; 2015.

[36] Tang D, Macosko CW, Hillmyer MA. Thermoplastic polyurethane elastomers from bio-based poly (δ-decalactone) diols. Polymer Chemistry 2014;5:3231-7.

[37] Guan J, Sacks MS, Beckman EJ, Wagner WR. Synthesis, characterization, and cytocompatibility of elastomeric, biodegradable poly (ester- urethane) ureas based on poly (caprolactone) and putrescine. Journal of biomedical materials research 2002;61:493-503.

[38] Grad S, Kupcsik L, Gorna K, Gogolewski S, Alini M. The use of biodegradable polyurethane scaffolds for cartilage tissue engineering: potential and limitations. Biomaterials 2003;24:5163-71.

[39] Lipik V, Widjaja L, Liow S, Venkatraman S, Abadie M. Synthesis of biodegradable thermoplastic elastomers (BTPE) based on epsilon-caprolactone. Express Polym Lett 2010;4:32-8.

[40] Uhrig D, Mays JW. Experimental techniques in high- vacuum anionic polymerization. Journal of Polymer Science Part A: Polymer Chemistry 2005;43:6179-222.

[41] Hadjichristidis N, Iatrou H, Pispas S, Pitsikalis M. Anionic polymerization: high vacuum techniques. Journal of Polymer Science Part A: Polymer Chemistry 2000;38:3211-34.

[42] Choi WY, Lee CM, Park HJ. Development of biodegradable hot-melt adhesive based on poly-ε-caprolactone and soy protein isolate for food packaging system. Lwt-Food Science and Technology 2006;39:591-7.

[43] Elzein T, Nasser-Eddine M, Delaite C, Bistac S, Dumas P. FTIR study of polycaprolactone chain organization at interfaces. Journal of colloid and interface science 2004;273:381-7.

[44] Coleman M, Zarian J. Fourier- transform infrared studies of polymer blends. II. Poly (ϵ-caprolactone)–poly (vinyl chloride) system. Journal of Polymer Science: Polymer Physics Edition 1979;17:837-50.

[45] Sang L, Wei Z, Liu K, Wang X, Song K, Wang H, et al. Biodegradable radiopaque iodinated poly (ester urethane) s containing poly (ε- caprolactone) blocks: Synthesis, characterization, and biocompatibility. Journal of Biomedical Materials Research Part A 2014;102:1121-30.

[46] Shin EJ, Jeong W, Brown HA, Koo BJ, Hedrick JL, Waymouth RM. Crystallization of cyclic polymers: Synthesis and crystallization behavior of high molecular weight cyclic poly (ε-caprolactone) s. Macromolecules 2011;44:2773-9.

[47] Tuba F, Oláh L, Nagy P. Towards the understanding of the molecular weight dependence of essential work of fracture in semi-crystalline polymers: A study on poly (ε-caprolactone). Express Polymer Letters 2014;8.

[48] Del Valle L, Díaz A, Royo M, Rodríguez-Galán A, Puiggalí J. Biodegradable polyesters reinforced with triclosan loaded polylactide micro/nanofibers: Properties, release and biocompatibility. Express Polymer Letters 2012;6:266-82.

Table 1. Molar Ratios of Reactants for Ring opening polymerization

experiment	**name**	**reaction mass (g)**	**[CL]:[BDO[a]]:[SO]**	**[CL] (mol/kg)[b]**	**[BDO] (mol/kg)**	**[SO] (mol/kg)**
1	PCL-Diol-3500	10	$31:1:1.1\times10^{-3}$	8.53	0.278	3×10^{-4}
2	PCL-Diol-8300	10	$73:1:2.5\times10^{-3}$	8.65	0.119	3×10^{-4}
3	PCL-Diol-16200	10	$142:1:5\times10^{-3}$	8.7	0.061	3×10^{-4}

[a] Mole of each particular alcohol.

[b] Mole of reagent per kilogram of reaction mass.

Table 2. Characteristic infrared bands of PCL [43-45]

Position (cm^{-1})	**Vibrator**
3300-3750	OH stretching
2949	Asymmetric CH_2 stretching
2865	Symmetric CH_2 stretching
1727	Carbonyl stretching
1293	C–O and C–C stretching in the crystalline phase
1240	Asymmetric COC stretching
1190	OC–O stretching
1170	Symmetric COC stretching
1157	C–O and C–C stretching in the amorphous phase

Table 3. Molecular and thermal properties of PCL-diols

Sample ID	**M_n**	**M_w**	**PDI**	**T_{cs}(°C)**	**T_c (°C)**	**T_m (°C)**	**ΔH_c ($J.g^{-1}$)**	**ΔH_m ($J.g^{-1}$)**	**X_c**
PCL-Diol-3500	3650	3803	1.042	29.43	27.48	57.60	102.79	127.00	17.8
PCL-Diol-8300	8352	8807	1.054	35.32	32.27	55.24	91.92	119.60	20.35
PCL-Diol-16200	16249	18956	1.167	29.39	27.39	45.52	112.37	131.07	13.75

Scheme 1. The proposed mechanism of the ring-opening polymerization of ε-caprolactone.

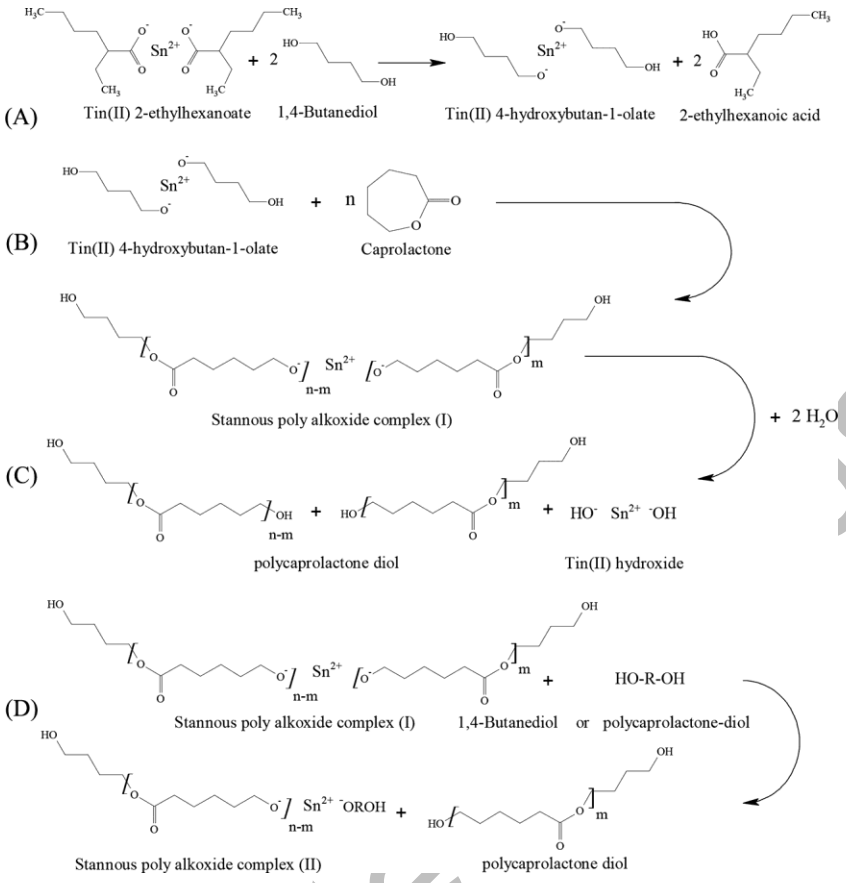


Figure 1. Infrared spectrums of PCL-diols in the region of 400–4000 cm^{-1}.

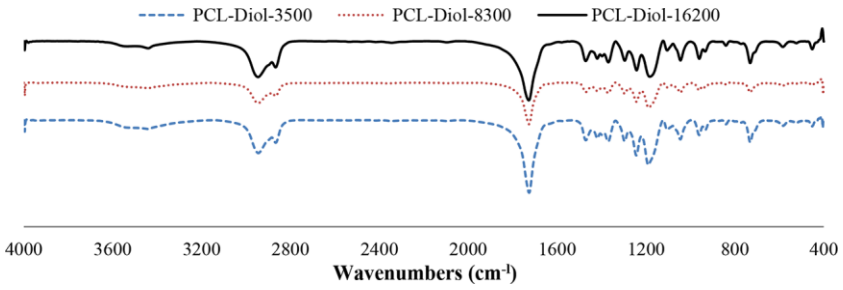


Figure 2. Crystallization and melting curves of PCL-diols in region -57°C to 100°C.

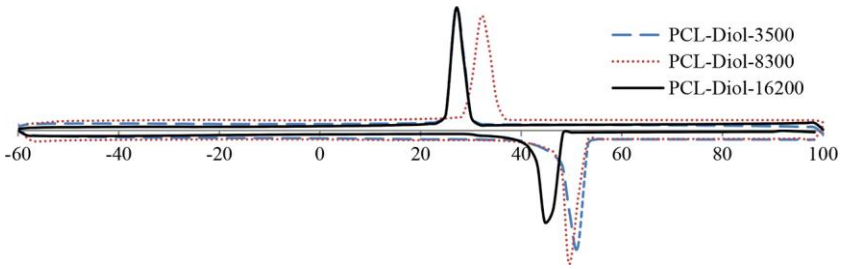


Figure 3. Stress strain curves of PCL-diols at room temperature. The modulus is taken as the slope of the linear part of the curve divided by the pre-test sample cross-section.

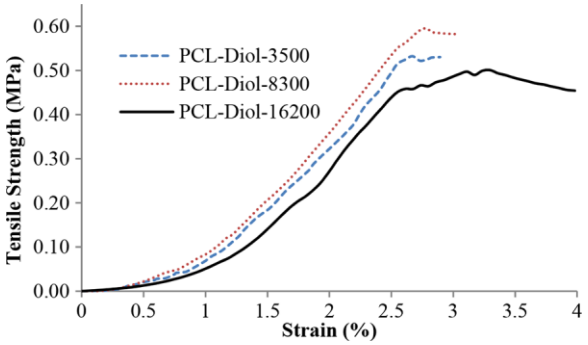
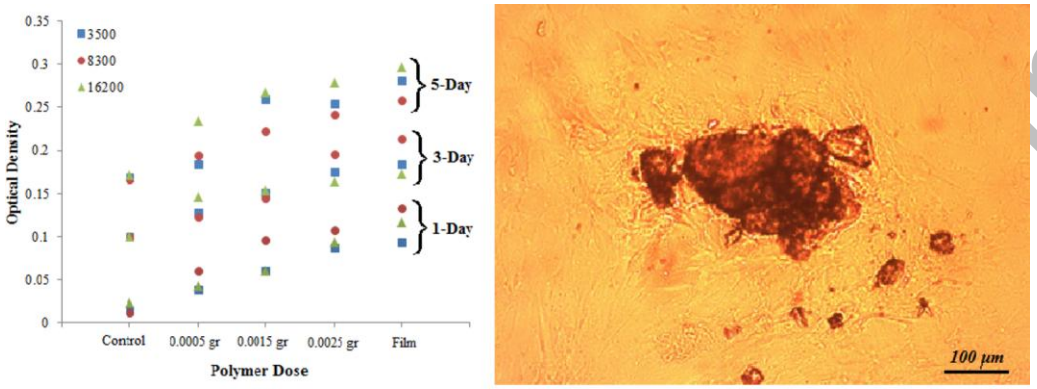


Figure 4. (a) Cytotoxicity of PCL-diols was measured by MTT assay with different molecular weights after Day 1, Day 3 and Day 5 by using mesenchymal stem cells. Each point is the average of three replications and their standard deviations are less than 5%. (b) Accumulation of mesenchymal stem cells around of PCL-diol particles.



(a) (b)

Figure 5. SEM image from accumulation of mesenchymal stem cells around of PCL-diol particles.

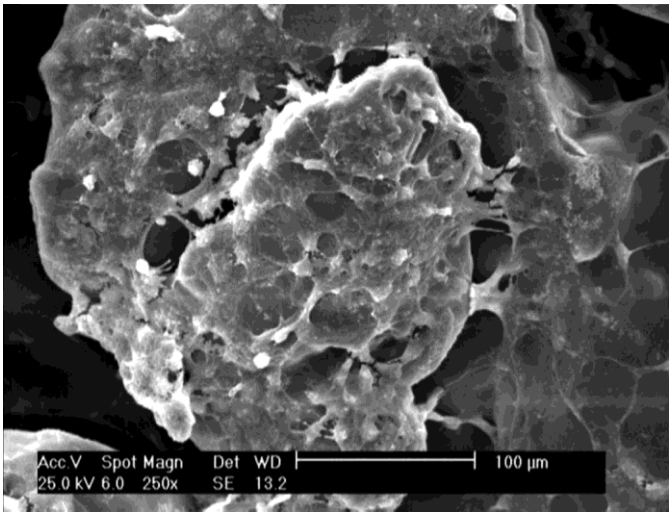


Figure 6. Relative cell viability PCL-diol-3500 (*a*), PCL-diol-8300 (*b*) and PCL-diol-16200 (*c*). Each column is the average of three replications results and their standard deviations are less than 5%.

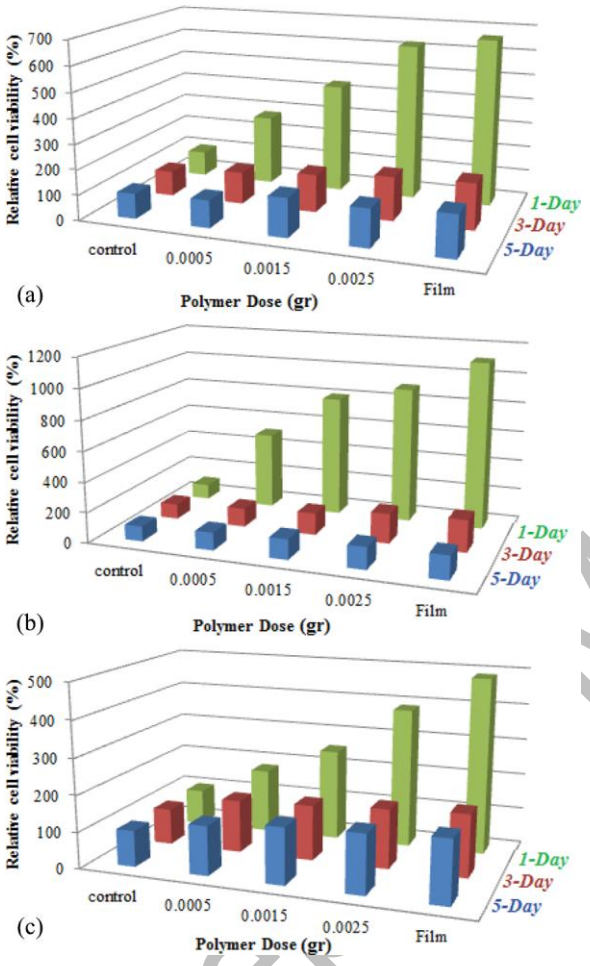


Figure 7. Optical microscope images for PCL-Diol-8300 as an example. Because PCL films were non-transmittance the images aren't recorded.