Synthesis and characterization of biodegradable poly(ether-ester) urethane acrylates for controlled drug release

Synthesis and Characterization of Biodegradable Poly(ether-ester) Urethane Acrylates for Controlled Drug Release

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Abstract

Three polyether-ester triblock diols, with various molecular weights, were synthesized from ε-caprolactone and polyethylene glycol and used, with diisocyanates, as soft segments for the preparation of polyurethane acrylate oligomers. The polyurethane acrylates were used to generate cross-linked polyurethane films *via* UV initiated polymerization with and without cargo incorporation. Degradation experiment indicated that in PBS/H_2O_2/$CoCl_2$ the membranes degraded rapidly compared to PBS alone or with lipase. The polyurethane membrane loaded with the antibiotic tetracycline, demonstrated prolonged release over 200 h, suggesting that the polymers could be used as an implant coating for controlled drug release.

Keywords：Poly(ether-ester)；Polyurethane acrylates；Biodegradable；Controlled drug release; Tetracycline

1. Introduction

Microspheres of polycaprolactone (PCL) and its copolymers are widely used in biomedical applications, especially in drug delivery, due to their excellent biodegradability, biocompatibility and drug releasing ability [1-2]. Many different types of PCL copolymers can be made including PCL homopolymers, PCL based amphiphilic copolymers, and polymers where PCL is used as a soft segment of a polyurethane. PCL homopolymers are a family of commonly used drug carriers, typically synthesized via ring-opening polymerization of ε-caprolactone (ε-CL) catalyzed by various initiators [3], however PCL homopolymers degrade very slowly under aqueous conditions due to their hydrophobicity which offers poor water permeation abilities. Modification of PCL to improve its water affinity and degradation rate can be achieved using PEG segments [4]. PCL based amphiphilic block copolymers typically display better water compatibilities than PCL

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homopolymers and can be prepared by conjugation with hydrophilic materials such as polyethylene glycol or polyacrylates [5]. These PCL amphiphilic copolymers are versatile building blocks that can be used to make a variety of multi-functional polymeric materials which can self-assemble into nano structures such as vesicles and micelles [6,7] as well as microspheres [8]. Upon incorporation of these functional groups polymers have been shown to be responsive to environmental stimuli such as temperature [9,10], pH [11,12], and reduction [13,14]. They can form matrices such as films, fibers and scaffolds for drug release [15] as well as injectable or oral vessels for controlled targeted drug delivery [16]. PCL polymers have also been used as soft segments in polyurethanes that have been used in drug delivery due to their non-toxic degradation products. Thus ophylline was encapsulated, for controlled drug release, within polyurethane microspheres composed of PCL and starch as the soft segments, with 4,4'-diphenylmethane diisocyanate (MDI) and 1,4-butanediol as the hard segments [17] with drug release dependent on the dissolution and diffusion of the drug as well as degradation of the polymer.

Biomaterials play an important role in many medical devices including as coatings for urinary catheters, or other implant devices, where they need to display both biocompatibility and anti-bacterial abilities. To generate anti-bacterial capability polymeric medical devices have incorporated many features, including metal ions (e.g. Ag^{+}), quaternary ammonium salts, antibiotics or PEG. Antibacterial polyurethane-based materials have been used as coatings for implant devices to increase biocompatibility and reduce inflammation. Basak [18,19] developed porous polyurethane films (using poly(ether-ester) diols obtained by reacting PEG400 with lactate and 2,4-toluene diisocyanate (TDI)) loaded with antibiotics (such as rifampicin, gentamicin and ciprofloxacin). The primarily release mechanism for the hydrophilic drugs being mainly related to diffusion while the release of lipophilic drugs was controlled mainly by polymer degradation.

Mândru [20] synthesized a poly(ester-ether urethane) using poly(butylene adipate) diol and PEG as soft segments and MDI together with 1,4-butanediol (BDO) as hard segments, from which the antibiotic rifampicin was released in a controlled manner depending on the molar concentration of urethane groups in the polymer chains as well as the surface morphology of the polyurethane membranes.

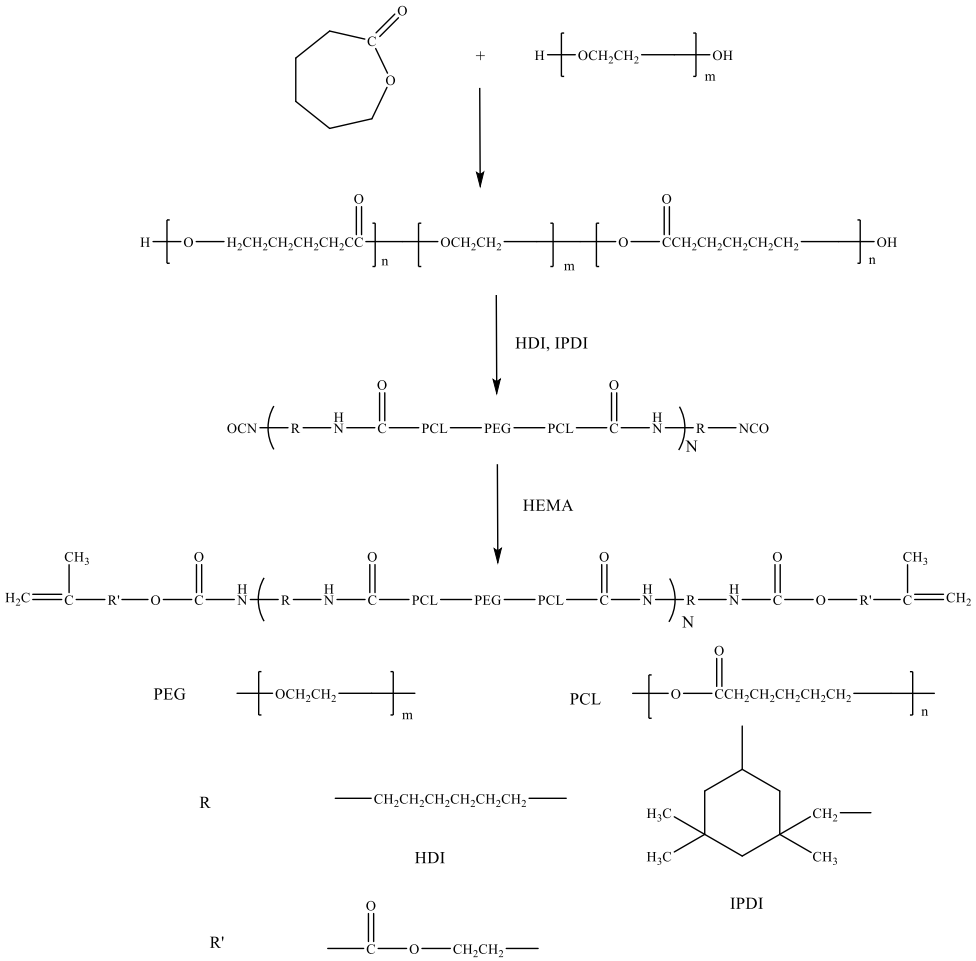
Here polyurethane acrylates that contained as a soft segment a block copolymer of polycaprolactone-poly(ethylene glycol)-polycaprolactone (PCL-PEG-PCL) were investigated as a biodegradable drug release vehicle. Polymers of differing

composition were synthesized by ring-opening polymerization of ε-caprolactone in the presence of PEG400 which was subsequently reacted with diisocyanates followed by hydroxylethyl methacrylates to give polyurethane acrylates (PUAs). By UV-curing the PUAs were polymerized to form cross-linked elastomers. Their properties and drug release profile from the cross-linked PUAs were studied.

2.Materials and Methods

2.1. Materials

1,6-Hexamethylene diisocyanate (HDI), isophorone diisocyanate (IPDI) and hydroxylethyl methylacrylate (HEMA) were purchased from Aladdin. ε-Caprolactone, was bought from Heowns Biochem Technologies LLC，and dried with CaH_2 for 24 h at room temperature and then distilled under vacuum before use. A solution of organo-bismuth (20 %) was purchased from Xianju Fusheng Compound Material Co. Ltd. PEG (M_w400), from Shanghai Ling feng Reagent Co. Ltd., was dried at 100^{o}C under vacuum for 2 h to remove residual water before use. Stannous octoate ($Sn(Oct)_2$) and 1-hydroxycyclohexyphenylketone (PI 184) were obtained from Sinopharm Chemical Reagent Co. Ltd. Toluene was dried by stirring with CaH_2 for 24 h before distillation and stored over 4 Å molecular sieves. The broad-spectrum antibiotic tetracycline (as the drug model) was purchased from Energy Chemical. All reagents were used as received unless mentioned otherwise. The UV lamp (model SB-100P/FA (100 w)) was from Westbury, USA.



Scheme 1: Synthesis of the polyurethane acrylate oligomers

2.2 Methods

2.2.1 Synthesis of polycaprolactone-poly(ethylene glycol)-polycaprolactone block copolymer (PdiolX)

The triblock copolymer diols (Scheme 1 and Fig. 1) were synthesized using PEG (M_w400, 5 g) to initiate the polymerization of ε-caprolactone (20 g) with $Sn(Oct)_2$ (0.3 wt%) as a catalyst in toluene (1 mL) at 130 ^oC for 24 h under stirring to obtain the triblock copolymers. To synthesize the copolymer diols with various molecular weights, the following molar ratios of PEG400 and ε-caprolactone were used (1/5, 1/14, 1/23 respectively) [21]. These ratios were used to control molecular of the soft segment (polymer diol) keeping it between 1000-3000 Da.

The products obtained were precipitated with excess cold hexane and ethyl ether respectively followed with centrifugation (1500 rpm) and drying in a vacuum oven at 50 ^oC for 24 h. The PCL-PEG400-PCL triblock copolymers are abbreviated as PdiolX where X represent the molecular weight of the triblock poly(ether ester).

2.2.2 Synthesis of PUAs

Polymers were synthesized using a two-step polymerization approach with a 2:1:2 ratio of diisocyanate (HDI or IPDI), PdiolX triblock copolymer and HEMA (as a capping agent) [22]. PdiolX (0.02 mol), toluene (4 mL), diisocyanate (0.04 mol) and $Sn(Oct)_2$ (0.05 g0.3 %wt) were added into the reactor and the reaction was carried out at 80 ^oC under dry nitrogen atmosphere for 3 h to obtain the isocyanate terminated polyether-ester diols. The solution was cooled to room temperature and HEMA (0.04 mol) was added dropwise under stirring. The reaction was heated to 80 ^oC under nitrogen and stopped (> 4 h) when the NCO groups of the polyurethane (at 2270 cm^{-1}) had disappeared as monitored by FTIR. The PUAs were precipitated following the addition of excess ethyl ether and collected by centrifugation (1500 rpm). The products were dried in a vacuum oven at 40 ^oC for 24 h to eliminate residual solvent.

2.2.3 Preparation of cross-linked PUA films

3 g of PUA were dissolved in DMF (1.5 mL, 200 %wt/v), followed by the addition of photo initiator (1 wt%，PI 184) and the solution stirred until homogeneous. The mixture was poured into glass molds previously coated with 1H, 1H, 2H, 2H-perfluorooctyl dimethyl chlorosilane (10 cm × 6 cm × 0.05 cm). The solution was exposed to UV light for 20 min and the films were placed in an oven at 50 ^oC for 4 h. The films were peeled off and washed in acetone for 12 h and then dried in a vacuum oven at 40 ^oC for 24 h. The films were cut into 10 mm × 10 mm samples and were approximately 0.05 cm (0.5 mm) thick.

2.2.4 Drug trapping in the cross-linked PUA films

The cross-linked PUA films (10 mm × 10 mm × 0.5 mm) were immersed into 8 mL of a THF solution of tetracycline (1 mg/mL) for 12 h. The swollen films were wiped with filter paper and dried in a vacuum oven at 40 ^oC for 6 h. The amount of tetracycline loaded in the polymer films were determined by soaking the films in THF (8 mL) for unloading under sonication for 1 h before the THF with the drug quantified/analyzed by UV/Vis spectrometery and compared to a standard concentration curve of tetracycline in THF.

2.3 Polymer characterization

^1H NMR spectra were recorded on a Bruker Avance NMR (400 MHz) with $CDCl_3$ as the solvent and tetramethylsilane (TMS) as the internal standard. Number average molecular weight (M_n), weight average molecular weights (M_w) and polydispersity index (PDI) of the triblock copolymer diols and PUAs were determined using gel permeation chromatography (GPC) (Waters Alliance 515). THF was used as the eluent at a flow rate of 1.0 ml/min at 20 °C, with molecular weights calibrated using

polystyrene standards.

Differential scanning calorimetry (DSC) was performed using a Perkin-Elmer DSC 800 under nitrogen at a constant heating rate of 10 °C/min. Samples were first heated from room temperature to 120 °C and held isothermally for 5min, followed by cooling from 120 to -75 °C and maintained at this temperature for 10min. Finally, the second heating was carried out from -75 to 120 °C. Sample weight for all measurements was in the range of 5-10 mg. X-ray diffraction analysis (XRD) was used to evaluate the crystallinity of the cross-linked PUA films with a STADI P diffractometer (STOE, Germany). The X-ray source was Cu/Kα radiation, powered at 40 kV and100 mA with a radiation wavelength of 1.542 Å. The scattering angle (2θ) ranges from 5 to 60° and was scanned at 2°/min. Dynamic mechanical analysis (DMA8000) was used to characterize the storage modulus and dissipation factor in corresponding to temperature. It was carried out in a tension mode at a single frequency of 1Hz, 0.04% strain and a heating rate of 4 ^{o}C /min in the range of -100 to 150 ^{o}C.

Contact angle measurements were performed using a JC2000D1 (Xiamen Maikailun Co., Ltd. China) equipped with a camera for imaging the test drops on the sample surfaces. The images were subsequently analyzed and calculated with the supplied software. Water was used as solvent for contact angle analysis at room temperature (~20 ^{o}C). Static and dynamic contact angles were measured with test interval of 5 min for the latter. For each sample the result was the average of four measurements. The swelling of a sample was measured by weighing the weight change of membrane(10 mm × 10 mm × 0.5 mm) before and after soaking in distilled water for 6, 24, 48 and 72 h, and in THF solution for 6 and 24 h in an incubator at 37^{o}C with a shaking speed of 60 rpm. At periodic intervals, the sample was removed from water or THF and wiped with filter paper to remove solvent from the surface of the film before weighing. Swelling of the samples was calculated by using following formula (1):

$$\text{Swelling (\%)} = \frac{W_a - W_b}{W_b} \times 100\% \quad (1)$$

Where, W_a and W_b are the mass of the sample after and before soaking in water or THF, respectively. The result of swelling was reported as an average of four replicates.

2.4 In vitro degradation

The degradation properties of the cross-linked PUA films were analyzed *in vitro* by measuring the weight loss over time in specific degradation solutions. Hydrolytic degradation was carried out in phosphate buffered saline (PBS) (0.01 M PBS with 0.02 % NaN_3, pH 7.40), enzymatic degradation was carried out in PBS buffer solution with 1 mg/mL lipase. Oxidative degradation was carried out in PBS buffer solution with H_2O_2 (20 wt%) and $CoCl_2$ (0.01 M). $H_2O_2/CoCl_2$ oxidative solution can

effectively generate hydroxyl radicals and hydroperoxy radicals which attack the polyether or polyester to cause degradation [23-24]. Each sample was placed into an individual vial containing 8 mL of the degradation solution, and then incubated at 37 ^{o}C with constant shaking (60 rpm). At one-week intervals, the sample was cleaned with deionized water for 6 h and dried in a vacuum oven at 50 ^{o}C for 24 h before being weighed. The weight loss was calculated using the following equation (2):

$$\text{Weight loss}(\%) = \frac{W_b - W_a}{W_b} \times 100\% \quad (2)$$

Where, W_b and W_a are the weights of the sample before and after degradation. The result for weight loss was reported as an average of four replicates.

2.5 Drug release and analysis

The stock drug solution was prepared by dissolving 4 mg tetracycline in 20 mL PBS or THF and used to prepare diluted solutions at 0.1, 0.05, 0.025, 0.01, 0.005 and 0.001 mg/mL. The absorbance of tetracycline was measured at 361 nm and the standard calibration curve was obtained by plotting the absorbance against the corresponding concentration of tetracycline solution (see Support Information). The soaked film was dried in a vacuum oven at 40 °C for 24 h.

To analyze the drug release profiles, the drug-loaded polymer films were placed in a dialysis bag and placed in a glass vial containing 8 mL of PBS. Periodically, the PBS solution was removed for absorbance measurement. Antibacterial assays (using *E. coli*) were performed to test the antibacterial properties of the polymer films with and without loaded drug.

E. coli were cultured for 12 h in a liquid medium (NaCl 5 g, beef extract 5 g and tryptone 10 g and water 1 L) at 37 ^{o}C. 100 μL of the cultured bacterial solution was inoculated on each sterilized agar plate. The cross-linked PUA films with and without loaded drug were placed on top of the agar plate and incubated at 37 ^{o}C for 24 hours. The areas without bacteria around the copolymer films (inhibition zone) were recorded.

3 Results and discussion

3.1 Characterization of triblock copolymer diols

The molecular weight and chemical structure of triblock copolymer diols were determined by ^{1}H NMR analysis in $CDCl_3$. As shown in Figure 1, the chemical shifts at 4.08 ($HOCH_2$-) and 2.30 (-CH_2COO-) correspond to the protons on the PCL units. The chemical shift at 3.64 (-OCH_2CH_2O-) can be assigned to the protons on the PEG units. Based on the intensity of peak a and b, the number of repeating units (n) of caprolactone and the molecular weight of the diols can be calculated [25] (Table 1, SI equation 1). The molecular weights and polydispersity (PDI) of the triblock

copolymer diols were also determined by GPC (Table 1), with the molecular weights successfully tailored by adjusting the ratio of ε-CL to PEG400. The results indicate that the $M_{n(NMR)}$ of the synthesized diols (see Figure 1) were close to the theoretical molecular weight-while GPC analysis gave PDIs of 1.4 to 1.6.

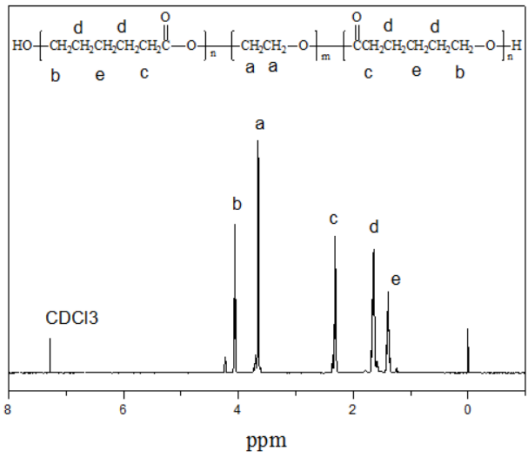


Figure 1 ^{1}H-NMR spectrum of the synthesized triblock copolymer diol (Pdiol2000) used to calculate the molecular weight of the polymer.

Table 1 Molecular weight of triblock copolymer diols

Triblock copolymer diols	**CL: PEG**	**$M_{n(NMR)}$**	**GPC**			**Yield(%)**
	（mol:mol）		**M_n**	**M_w**	**PDI**	
Pdiol1000	5:1	869	1500	2100	1.42	83.3
Pdiol2000	14:1	2053	3300	5500	1.66	85.3
Pdiol3000	23:1	3162	5000	7600	1.54	82.9

3.2 PUA oligomer characterization

The molecular weights and polydispersity of the PUAs were determined by GPC (Table 2). The molecular weight of the PUAs ranged from 4000 to 14,000, increasing significantly as the molecular weight of the copolymer diols increased, with PDIs 1.5-1.7. Since the stoichiometry of the monomers (HDI (or IPDI)): triblock copolymer diols: HEMA) was 2:1:2, the polymerization degree of the PUA was about 3, with a structure of HEMA-((HDI or IPDI)-PCEC)$_3$-(HDI or IPDI)-HEMA. The oligomers were also analyzed with ^{1}HNMR and FTIR (Fig. S1, S2)

Table 2 Molecular weight and polydispersity of the PUAs

PUAs	M_n	M_w	PDI
Pdiol1000-HDI	4090	6400	1.57
Pdiol1000-IPDI	4100	6500	1.57
Pdiol2000-HDI	8800	14700	1.67

Pdiol2000-IPDI	8400	13100	1.56
Pdiol3000-HDI	13500	21600	1.60
Pdiol3000-IPDI	12400	21100	1.70

3.3 Structure and morphology of cross-linked PUA films

DSC was used to study the thermal behavior and microphase separation of the cross-linked PUA films and XRD was used to analyze the crystallinity of the polymers. The results suggest that Pdiol1000-HDI, Pdiol1000-IPDI and Pdiol2000-IPDI were all amorphous polymers with low glass transition temperatures and characteristic broad peaks, which agreed with literature [26] (Table 3, Fig. 2). XRD results of PUAs showed that Pdiol3000-HDI had sharp crystallization peaks with relatively high crystallinity 51 %, corresponding to the melting peak at about 40 ^{o}C on the DSC curve. Pdiol2000-HDI and Pdiol3000-IPDI had only minor spikes on XRD indicating incomplete crystallization, which were consistent with the melting peaks and obvious glass transitions temperatures of amorphous parts in the polymer films on DSC curves.

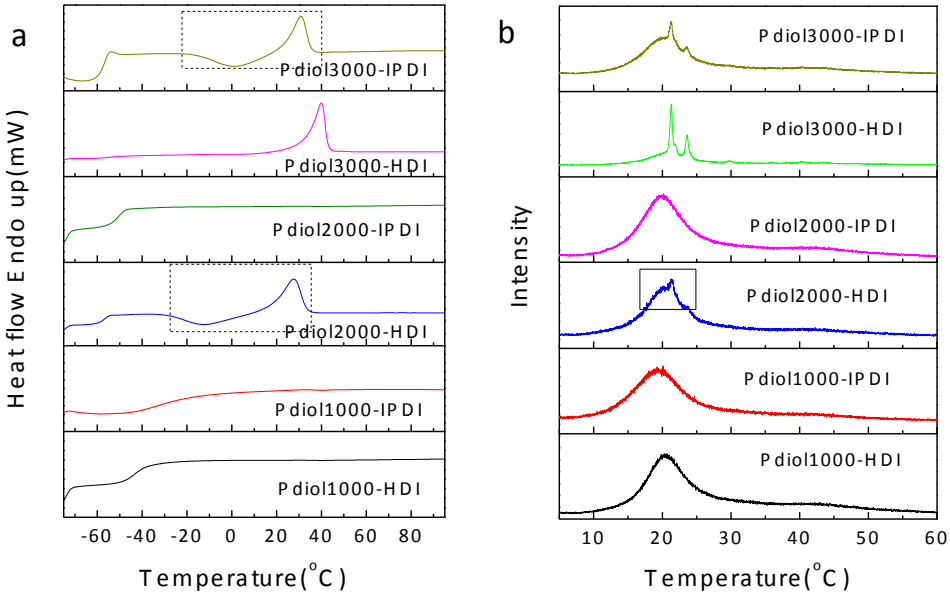


Figure 2 The morphology analysis of cross-linked PUA films: (a) DSC heating curves. Data were collected from the reheating run with scanning temperature from -75 to 120 ^{o}C with 10 ^{o}C/min. (b) XRD spectra. The polymer films were cut into 10 mm × 10 mm samples for XRD measurement. The measurement was carried out under room temperature (20 ^{o}C) with the scanning angle (2θ) from 5 to 60° at a scan step of 0.02°.

Pdiol2000-HDI, Pdiol3000-HDI and Pdiol3000-IPDI showed low Tg's and Tm's (Table 3). Additionally Pdiol2000-HDI and Pdiol3000-IPDI also exhibited cold

crystallization peaks. It has been reported that when the molecular weight is less than 2000 g/mol, PCL is difficult to crystallize [27]. But the symmetrical structure of Pdiol2000-HDI changed this and improved the formation of the crystals. On the other hand, despite its long PCL segments, the reason of higher cold crystallization temperature (Tc=0 °C) of Pdiol3000-IPDI than the Pdiol2000-HDI was probably due to the IPDI, which acts as a hard segment with asymmetric structure to suppress the cold crystallization of PCL at lower temperature in the cross-linked PUA films.

Table 3 Thermal properties of cross-linked PUA films

Sample	Tg(°C)	Tm(°C)	ΔH(J/g)	Crystallinity（%）
Pdiol1000-HDI	-427	--	--	--
Pdiol1000-IPDI	-30.1	--	--	--
Pdiol2000-HDI	-57.1	21.5	5.1	6.6
Pdiol2000-IPDI	-51.2	--	--	--
Pdiol3000-HDI	-55.2	40.0	43.4	50.9
Pdiol3000-IPDI	-57.5	30.8	21.7	25.5

Microphase separation that occurred due to crystallization in the cross-linked PUA films could be detected using DMA (Fig. 3 and Table 4). The storage modulus of the Pdiol3000-HDI showed larger storage modulus than that of Pdiol1000-HDI (which had the second highest overall crosslink density compared to the other samples) due to the highest crystallinity (50.9 %, Table 3) among all of the polymers. In the same reason the Pdiol3000-IPDI showed also higher storage modulus than the amorphous Pdiol2000-IPDI.

The Pdiol3000-HDI and -IPDI showed broad peaks on tanδ curves indicating there were at least two steps of chain relaxation [26], including relaxation of soft and hard segments respectively (Fig. 3b, d). The peaks at low temperature showed glass transition of the soft parts on the polymer chains, corresponding to the first step decreasing of the storage modulus. While those peaks at the higher temperature were glass transitions of hard segments including urethane acrylate and the melting of the small crystals, corresponding to the second step of the storage modulus reduction. Other polymers did not show the similar obvious broad peaks on tanδ curves because of either high crosslink density or low crystallinity. However, the trend of the Tg of the polymers is increasing with the crosslinking density and the Tg was normally higher when IPDI was used.

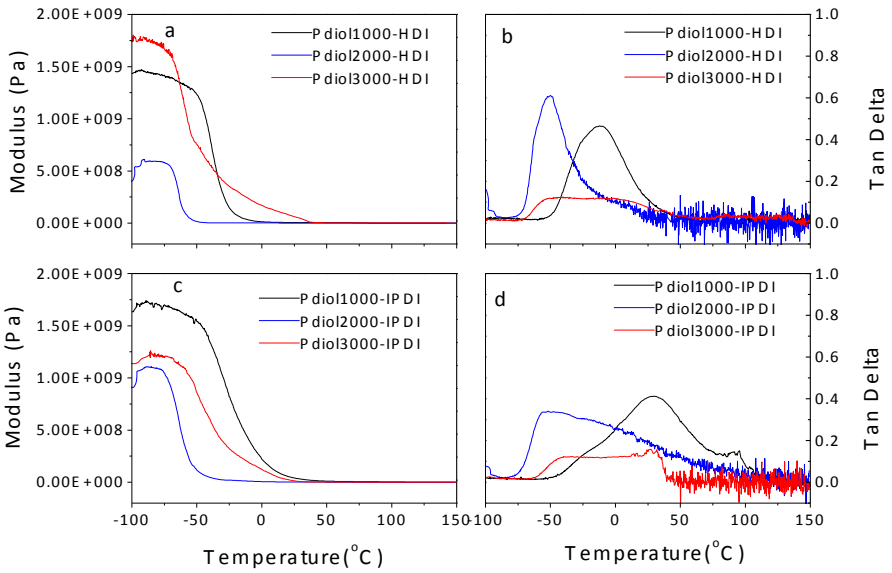


Figure 3 Dynamic mechanical temperature sweep curves of cross-linked PUA films at single frequencies of 1Hz with the temperatures scanned from −100 to 150 °C with a heating rate of 4 °C/min and 0.04 % strain. (a, c) Storage modulus and (b, d) loss factor (tan Delta).

Table 4 Tg, modulus and cross-link density of cross-linked PUA copolymers based on DMA measurements

Sample	Tg(°C)	$E_N^0 \times 10^6$ (N/m^2)	ν_e (mol/m^3)
Pdiol1000-HDI	-14.0	4.5	555.3
Pdiol1000-IPDI	28.3	10.8	1338.1
Pdiol2000-HDI	-51.6	1.3	159.0
Pdiol2000-IPDI	-56.5	1.6	200.1
Pdiol3000-HDI	-45.1/-14.1	0.6	73.7
Pdiol3000-IPDI	-40.6/24.4	1.1	138.9

Notes: Average molecular weight between cross-linking sites, $M_c = 3\rho RT / E_N^0$; Cross-link density of cross-linked PUA polymers, $\nu_e = \rho / M_c$, therefore $\nu_e = E_N^0 / (3RT)$, here T and R are respectively 323K and 8.314 m^{3}Pa/mol K.

3.4 Hydrophilicity of polymers

Polymer hydrophilicity is an important factor that affects the polymer degradation and drug delivery properties. Contact angle and swelling are measurements of the hydrophilicty of a surface and the bulk properties of the polymer.

Results indicate that a higher molecular weight of PCL lead to more hydrophobic cross-linked PUA films with larger contact angles (Table 5). The Pdiol1000-HDI was the most hydrophilic material among all the polymers with instant contact angles greater than the corresponding dynamic contact angles (measured after dropping

water on the surface for 5 min) due to the diffusion of the water along the polarized part of the polyurethane chains, which causes the surface rearrangement of the cross-linked PUA [28].

Table 5 Water contact angles of the cross-linked PUAs

(Standard deviation errors, n=4)

Sample	5s	5min
Pdiol1000-HDI	63.3±5.1	35±2.6
Pdiol1000-IPDI	74.5±3.8	56.7±0.8
Pdiol2000-HDI	76.0±3.7	58.0±3.0
Pdiol2000-IPDI	75.5±3.9	57.2±4.5
Pdiol3000-HDI	77.5±1.7	60.5±2.6
Pdiol3000-IPDI	78.8±2.1	67.2±2.9

Swelling of the cross-linked PUA films as a result of solvent uptake in water and THF showed that the Pdiol1000-HDI had the highest water uptake of 9 % after 72 h, which was consistent with the water contact angle results which showed it to be the most hydrophilic polymer (Fig 4). Water uptake reduced with the increase in molecular weight of PCL because of its hydrophobic nature. However, the water uptake increased with longer soaking time in water for all the polymers (Fig 4a).

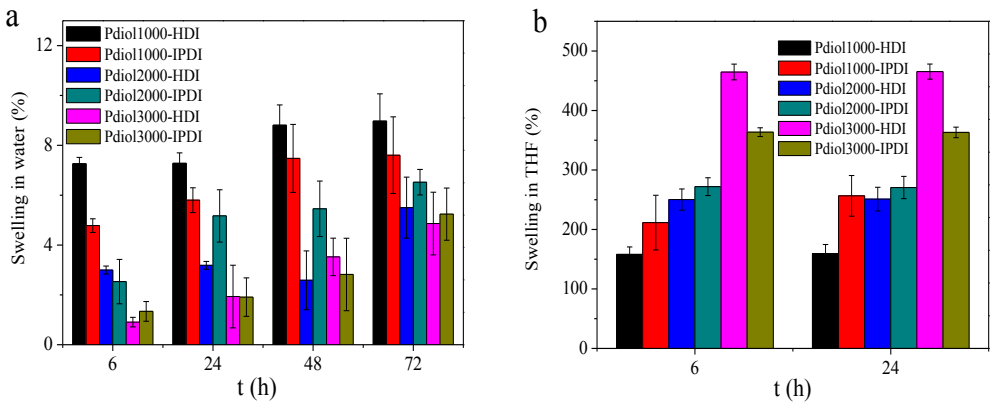


Figure 4 Swelling of cross-linked PUA films. （a）Swelling of cross-linked PUA films in water; （b）Swelling of cross-linked PUA films in THF. Errors are standard deviation, n=3.

The swelling in THF of the cross-linked PUA films were also measured (Fig 4b) at the time points of 6 and 24 h respectively. It showed that most of the polymers had reached a saturated swelling state after 6 h. The highest swelling ratio among all polymers was Pdiol3000-HDI reaching 465 % swelling in 24 h. However, Pdiol3000-IPDI showed only a swelling of 360 %. The reason might be the ring structure of the IPDI increased the cross-link density after polymerization (Table 3)

that suppressed the swelling of the polymer.

3.5 *In vitro* degradation

The biodegradation of cross-linked PUA films was investigated under different conditions including lipases and oxidative milieu as both, esterases and peroxides (Fig. 5), play important roles for biodegradation in *in vivo* systems [29].

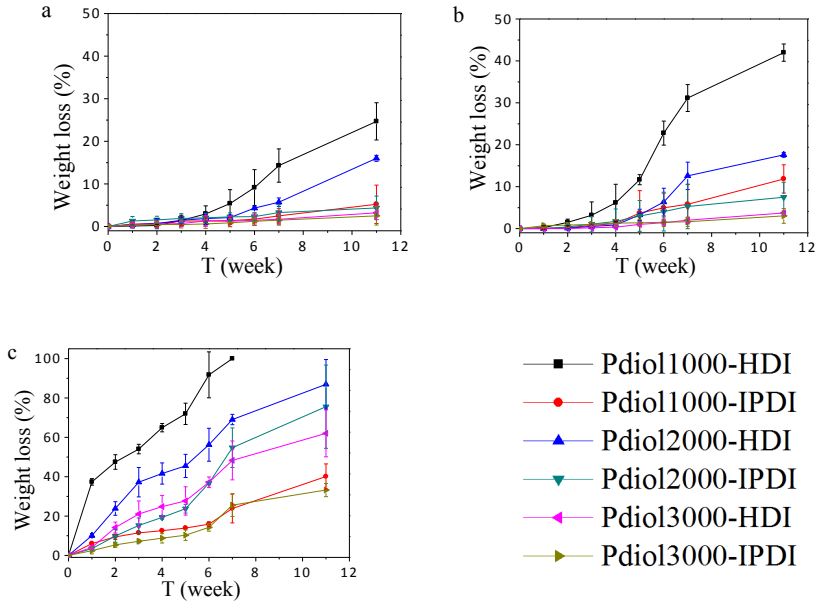


Figure 5 Degradation profiles of cross-linked PUA films in degradation solutions. (a) Hydrolytic degradation of polymers in PBS solution (pH=7.4); (b) Enzymatic degradation of polymers in lipase solution; (c) Oxidative degradation of polymers in H_2O_2 solution. Error bars represent mean standard deviations, n = 4.

The results of weight loss experiments indicate that the cross-linked PUAs degraded much more rapidly in H_2O_2 solution than the others (Fig 5c). Pdiol1000-HDI degraded fully after 7 weeks. Even the most hydrophobic Pdiol3000-IPDI degraded over 30% in 11 weeks. This means that these kinds of polymers were vulnerable to attacked by these free radicals. Christenson [30-31] proposed that the oxidative degradation of polyester urethanes is similar to that of polyether urethanes. The hydrogen peroxide produces hydroxyl and hydroperoxy radicals in water, via $CoCl_2$ mediated catalysis, attacking the hydrogen of the α-methylene group, leading to chain scission [24]. Lipase accelerated degradation of hydrophilic polymers was (as expected) more prominent than with hydrophobic ones (Fig. 5b), as enzymatic degradation happens mainly around water soluble domains on the polymer chains，such as found in Pdiol1000-HDI which lost about 45% by mass in 11 weeks (Fig. 5a). The degradation of hydrophobic polymers catalyzed by lipases was less significant.

Upon oxidative degradation in H_2O_2 solution for 5 weeks, the transparent cross-linked PUA films of Pdiol1000-HDI, Pdiol1000-IPDI and Pdiol2000-IPDI were observed to become rough, opaque and with cracks (Fig. S3). Especially Pdiol1000-HDI, which lost a large part of its weight and shrunk significantly. Surface damage was also discerned when they degraded in PBS and lipase solutions for the same period of time, showing stress lines and roughness on the surfaces, The opaque films of Pdiol3000-HDI and –IPDI however showed almost no change (via the naked eye). SEM images showed the porous type of character of the Pdiol1000-HDI polymer film after degradation in H_2O_2 solution (Fig. 6a, b).

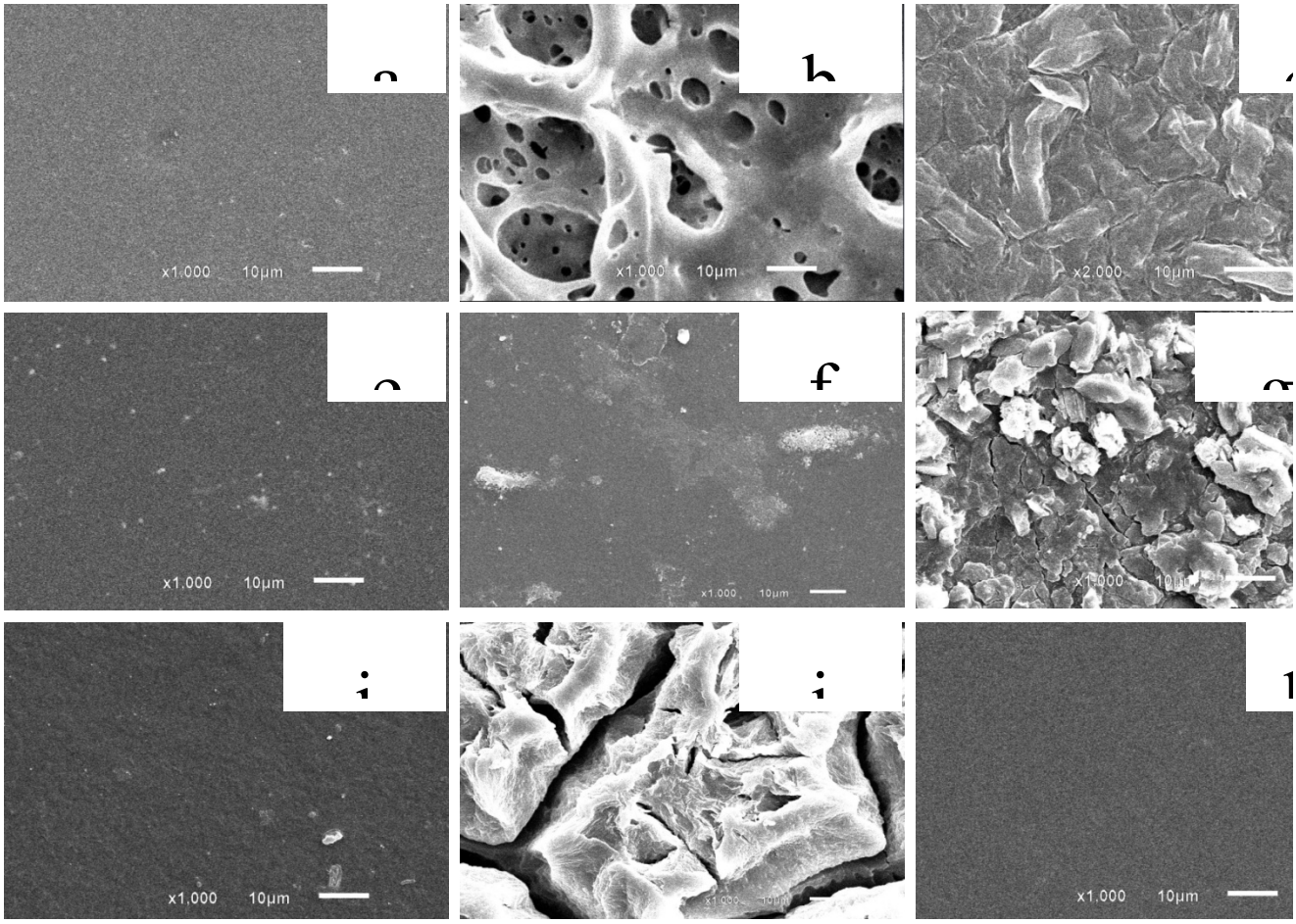


Figure 6 SEM images of the cross-linked PUA membranes before and after degradation experiments in PBS, PBS/lipase and PBS/H_2O_2/$CoCl_2$ solutions for 5 weeks. (a) Pdiol1000-HDI before degradation; (b) Pdiol1000-HDI after degradation in PBS/H_2O_2/$CoCl_2$ solution; (c) Diol2000-HDI before degradation; (d) Pdiol2000-HDI after degradation in PBS; (e) Pdiol2000-IPDI before degradation; (f) Pdiol2000-IPDI after degradation in lipase solution; (g) Pdiol2000-IPDI after degradation in PBS/H_2O_2/$CoCl_2$ solution; (h) Pdiol3000-HDI before degradation; (i) Pdiol3000-HDI after degradation in PBS; (j) Pdiol3000-HDI after degradation in PBS/H_2O_2/$CoCl_2$ solutions; (k) Pdiol3000-IPDI before degradation; (l) Pdiol3000-IPDI after degradation in PBS/H_2O_2/$CoCl_2$ solution. Scale bars in all SEM images are 10μm.

3.6 Drug release from cross-linked PUAs as long-term antibacteria releasing film

The drug was loaded onto cross-linked PUA films (10 mm × 10 mm× 0.5 mm) by immersing films in THF with tetracycline (1 wt%) for 12 h followed by removal of the solvent *in vacuum* at 50 °C. THF was used as it swells the polymer and dissolves the drug. The drug loading capacity of the polymer films was determined by washing the drug out of the films with THF (Table 6) showed that Pdiol3000-IPDI had the highest drug loading, while Pdiol1000-IPDI had the lowest.

Table 6 The drug loading capacity on cross-linked PUA films (n=4)

Sample	Loading capacity (μg/mL)
Pdiol1000-HDI	20.5±1.4
Pdiol1000-IPDI	14.0±2.9
Pdiol2000-HDI	25.7±0.4
Pdiol2000-IPDI	24.9±1.5
Pdiol3000-HDI	36.7±12.4
Pdiol3000-IPDI	43.6±5.5

The drug release profile of the cross-linked PUA films were carried out in PBS and cumulative release percentage is presented in Fig. 7. It was found that Pdiol2000-IPDI and Pdiol3000-HDI exhibited high levels of drug release, releasing 87 % and 58 % of the loaded drug respectively after 250 h. Pdiol3000-IPDI released 38 % of the loaded drug after 300 h and Pdiol1000-HDI released 35 %, while Pdiol1000-IPDI and Pdiol2000-HDI released only 14 % and 8 % of the loaded drug even after 300 h.

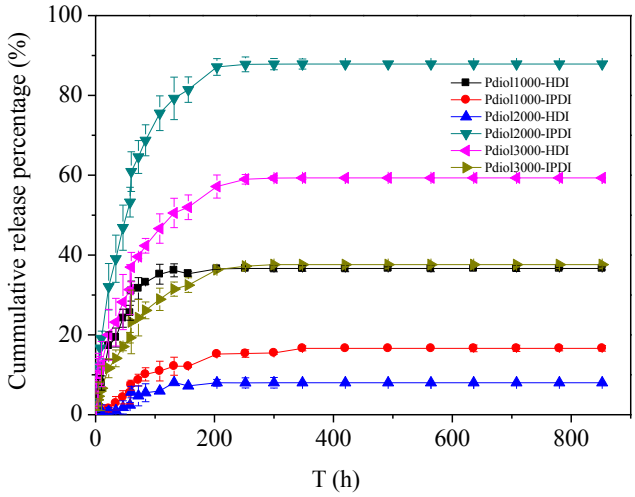


Figure 7 Drug releasing profile of cross-linked PUA films loaded with tetracycline in PBS. Errors are STDEV, n=3.

All polymers swelled to approximately the same level in water (see Fig. 4), likewise within 800 hours (approx. 5 weeks) there was still only limited degradation (≈5 % in PBS, see Fig. 5) suggesting that drug release is dominated by drug-polymer interactions. Thus more of the drug

will stay entrapped within the hydrophilic polymers (e.g. Pdiol1000) via H-bonding (tetracycline has a logp of -1.30). With the two polymers Pdiol2000IPDI and the Pdiol3000HDI (that show greatest release) we believe that interactions to trap the hydrophilic drug within the more hydrophobic environment are reduced, thus leading to enhanced drug release.

The tetracycline loaded cross-linked PUA films were applied to lawns of bacteria to test their anti-bacteria capacity (10 mm × 10 mm), releasing drug which diffused into the gel and prevented bacterial growth (Fig. 8). This zone diffusion antibiotic assay was used to augment the tetracycline release assays to prove that the drug released was active (non-degraded) and capable of killing bacteria at the released dose. It was also used to show that the control polymer was non-antibacterial in nature.

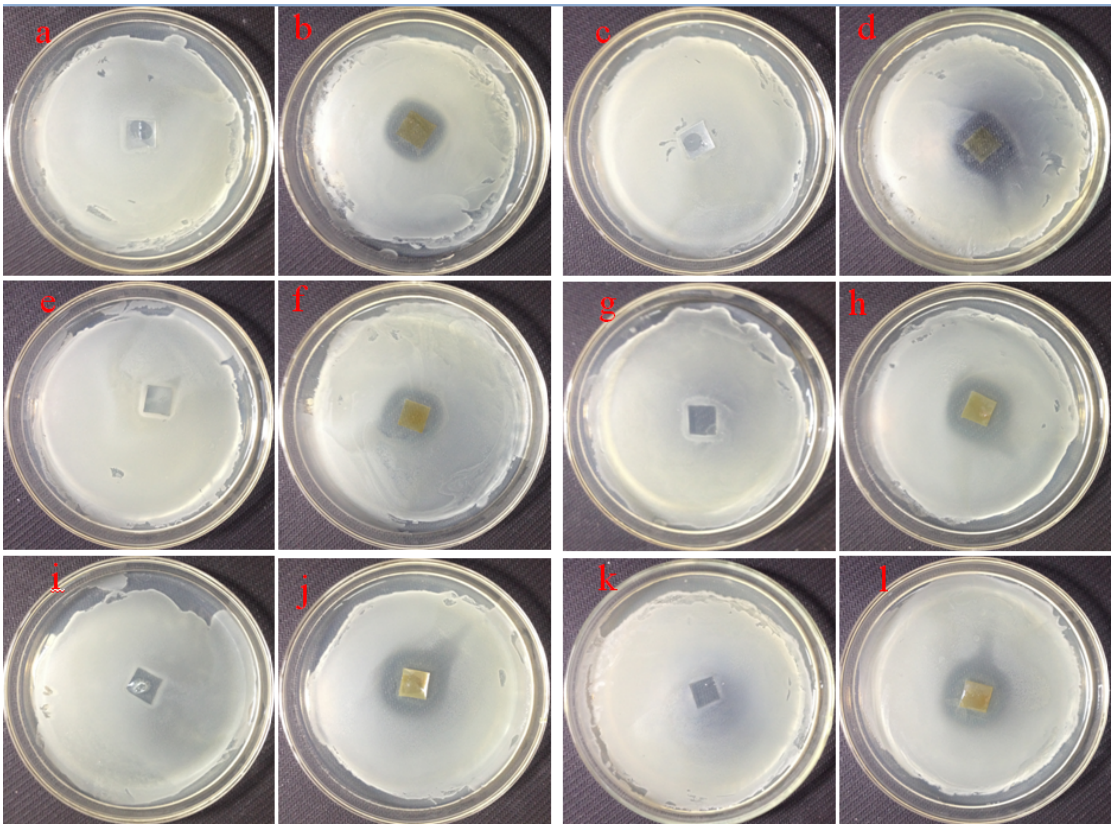


Figure 8 Images of inhibition zones of tetracycline loaded crosslinked PUA films incubated on lawns of bacteria for 24 h. (a) Pdiol1000-HDI without drug; (b) Pdiol1000-HDI with drug; (c) Pdiol1000-IPDI without drug; (d) Pdiol1000-IPDI with drug; (e) Pdiol2000-HDI without drug; (f) Pdiol2000-HDI with drug; (g) Pdiol2000-IPDI without drug; (h) Pdiol2000-IPDI with drug; (i) Pdiol3000-HDI without drug; (j) Pdiol3000-HDI with drug; (k) Pdiol3000-IPDI without drug; (l) Pdiol3000-IPDI with drug.

4. Conclusion

In this study, PCL-PEG-PCL triblock copolymer diols with various molecular weights were successfully synthesized by changing the PEG400/ε-CL ratio, and the PUA oligomers were obtained by reacting copolymer diols with HDI/IPDI and then

end-capped with HEMA. After UV-curing, cross-linked PUAs were prepared. By increasing the molecular weight of PCL segments (in copolymer diols), the crystallinity of the cross-linked PUA films increased and their swelling in water reduced. Experiments showed that the cross-linked PUA films degraded most rapidly in H_2O_2 solution. In the lipase solution, only the hydrophilic polymer films showed greater degradation than in PBS solutions. Degradation rates were also influenced by the structure of the polymer. Pdiol1000-HDI films showed the fastest degradation rates among all of the polymers in H_2O_2 solutions due to their excellent hydrophilicity, amorphous state and reduced cross-link densities. The cross-linked PUA films loaded with antibiotic drug tetracycline showed sustained drug release. This work demonstrates that these antibiotic loaded polymer films have the potential to be applied in medical devices such as in-dwelling catheters which are a major source of hospital infections, as well as coatings for other types of implants (e.g. metal-based implants) that can also result in chronic infections. Typically in these scenarios drugs need to be released in a sustained manner due continual bacterial insult and as such the materials we have designed, that allow controlled and long term drug release would find useful application.

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