Tetracycline Release from Bioerodible Hydrogels Based on Semiinterpenetrating Polymer Networks Composed of Poly(ε-caprolactone) and Poly(ethylene glycol) Macromer *In Vitro*

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SYNOPSIS

Poly(ethylene glycol) (PEG) macromers terminated with acrylate groups and semiinterpenetrating polymer networks (SIPNs) composed of poly(ε-caprolactone) (PCL) and PEG macromer were synthesized and characterized with the aim of obtaining a bioerodible hydrogel that could be used to release tetracycline HCl for local antibiotic therapy administered peroperatively. Polymerization of PEG macromer resulted in the formation of crosslinked gels due to the multifunctionality of macromer. Noncrosslinked PCL chains were interpenetrated into the crosslinked three-dimensional networks of PEG. Glass transition temperature (T_g) and melting temperature (T_m) of PCL in the SIPNs were inner shifted, indicating interpenetration of PCL and PEG chains. It was found that water content increased with increasing PEG weight fraction due to the hydrophilicity of PEG. Drug release can be controlled by weight fraction of PEG in the PCL/PEG SIPNs, concentration of PEG macromer in the SIPNs preparation, and the nature of PEG. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Drug release from biodegradable or bioerodible polymer matrices has been intensively investigated for the last decade.[1] One major advantage of using a biodegradable system is to eliminate surgical removal of an implanted delivery device after the delivery system is exhausted.[2]

The most thoroughly investigated and used bioerodible polymers are the poly(α-hydroxy esters), such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(LA-*co*-GA) that would degrade into naturally occurring substances.[3] Also, polyanhydrides,[4] poly(ortho esters),[5] and poly(α-amino acids)[6] have been developed.

Implantable delivery systems using the biodegradable polymers have been extensively investigated for peptide drugs,[7] anticancer therapy,[8] hormone therapy,[9] antihypertensive drugs,[10] and anesthesia.[11]

Recently, Mauduit et al. reported gentamycin release from the gentamycin/PLA blends for peroperative local antibiotic therapy to avoid secondary effects by the oral route and to reduce patient discomfort by the intramuscular route.[12-14] But none was based on tetracycline release from the bioerodible hydrogels for local antibiotic therapy administered peroperatively.

In this study, we report tetracycline release from a bioerodible hydrogel based on semiinterpenetrating polymer networks (SIPNs) composed of poly(ε-caprolactone) (PCL) and poly(ethylene glycol) (PEG) macromer. PCL is one of the biodegradable polyesters that have attracted attention in controlled

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drug delivery due to their nontoxicity. But the homopolymer itself is degraded very slowly when compared with PGA, PLA, and poly(GA-*co*-LA) copolymers. The biodegradability can be enhanced by copolymerization[15] or blends with a variety of hydrophilic polymers.[16]

We decided to incorporate PEG macromer into PCL chains because PEG macromer is a hydrophilic segment that can be used to enhance the biodegradability of PCL. Also, it may be expected that SIPNs composed of PCL and PEG macromer can be used to increase its compatibility more than the PCL/PEG blend.[17]

EXPERIMENTAL

Materials

PEGs with 1,500 and 7,500 molecular weights were obtained from Wako Pure Chemicals, Inc. PEG with molecular weight 20,000 and tetracycline hydrochloride were purchased from Sigma. All PEGs were α,ω-dihydroxy, and were purified by azeotropic distillation from benzene solution. PCL (MW 40,000) was obtained from Aldrich. 2-Hydroxy isobutyl phenol was kindly supplied by Kansai Paint Co. LTD. Acryloyl chloride was purchased from Reagent Chimica. All other chemicals used were reagent grade and used without further purification.

Synthesis of PEG Macromer

A total of 12 g of purified PEG 7.5K was added in 150 mL of benzene in a 500-mL, round-bottomed flask and was heated to 80°C in an oil bath. A total of 0.46 mL of triethylamine and 0.61 mL of acryloyl chloride were added to the flask and the reaction mixture was stirred for 3 h at 80°C. The reaction mixture was filtered to remove triethanolamine hydrochloride and the macromer was obtained by pouring the filtrate into *n*-hexane. Then, it was dried at 40°C under vacuum overnight. Other macromers were similarly synthesized.

Synthesis of Semi-PCL/PEG IPNs

The SIPNs were prepared by the simultaneous IPNs method. Ten microliters of the initiator solution (2-hydroxy isobutyl phenol) was added to the methylene chloride solution of PCL and PEG macromer (20 wt %, w/w). The mixture solution was irradiated using a low-density LWUV lamp (Toshiba Chemical Lamp FL 20 BL: wave range 300–400 nm, maximum intensity 360 nm) for 5 min and the solution was then evaporated to dryness at 4°C. The solid was further dried at 40°C under vacuum. Then, the prepared SIPNs were repeatedly washed with cold water for 1 day to remove unreacted PEG macromer.

Measurements

IR spectra were measured by a Bruker IFS-66 FTIR spectrometer. NMR spectra were obtained on a Bruker WP 80 SY FT-NMR spectrometer. The glass transition temperature (T_g) and melting temperature (T_m) were measured with a Mettler DSC-30 differential scanning calorimeter. The measurements were carried out in the range of −100–100°C under liquid nitrogen at a scanning rate of 10°C/min. Disks were fixed on an adhesive tape and coated with gold/palladium before observation by SEM using a JSM-35CK Jeol microscope.

Water Content

Dry SIPN films without drug were incubated in distilled water at 37°C. At preset time intervals, hydrated samples were weighed after blotting the surface water with filter paper. Water contents were calculated as $W_s - W_d/W_s \times 100\%$, where W_s and W_d are wet weight and dry weight of the SIPNs, respectively.

Tetracycline Loading

The desired amount of tetracycline hydrochloride was dispersed in a methylene chloride solution of PCL and PEG macromer. The dispersed solution was then exposed to the LWUV lamp for 5 min and the solution was then evaporated to dryness at 4°C. The solid residue was further dried at room temperature under vacuum for 1 day.

In Vitro Release

Cut disks of the drug loaded polymer were introduced into a vial with 5 mL of a phosphate buffer solution (PBS, 0.1*M*, pH 7.4). The mixture was stirred in a shaker whose temperature was maintained at 37°C. At predetermined time intervals, 5-mL aliquots of the aqueous solution were withdrawn and another 5 mL of PBS was put into the vial. The concentration of tetracycline HCl released was monitored using a UV spectrophotometer at 365 nm.

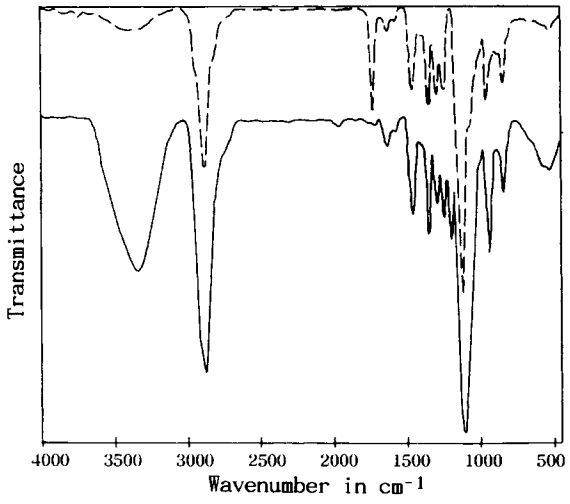

Figure 1 IR spectra of (—) PEG and (- - -) PEG macromer (MW of PEG, 1,500).

In Vitro Degradation

The cut dry SIPNs were equilibrated in PBS solution at pH 7.4 and incubated at 37°C. Weight loss was monitored gravimetrically at various intervals of time.

RESULTS AND DISCUSSION

FTIR spectra of PEG 1.5K and its PEG macromer are shown in Figure 1. The FTIR spectrum of the PEG showed an absorption band at 3,447 cm^{-1} due to the terminal hydroxyl group.[18] This band became weak in the PEG macromer due to acrylation. A new absorption was seen at 1,726 cm^{-1} in the PEG macromer due to the carbonyl bond of the acrylate group.[19] The band at 2,872 cm^{-1} was attributed to the C—H stretch[20] and was present in both polymers. Also, the NMR spectrum for PEG macromer showed small peaks for the three protons of the acrylate group at 4.8, 5.0, and 5.2 ppm (not shown in fig.). These results indicate that the terminal hydroxyl groups in the PEG precursor were converted to acrylate groups by a reaction with acryloyl chloride. Because PEG has two hydroxyl groups per molecule, the number of acrylic groups on the PEG is expected to be 2.

Water-soluble PEG macromer forms a crosslinked three-dimensional network upon free-radical polymerization. These PEG macromers underwent rapid photopolymerization, even in the presence of oxygen.[21] But UV curing time was 5 min to achieve complete gelation and a three-dimensionally crosslinked network. The gel content of 1.5K, 7.5K, and 20K PEG macromers were 78.1, 94.0, and 88.8 wt %, respectively (concentration of PEG macromer 30 wt %). Noncrosslinked PCL chains were interpenetrated into the crosslinked three-dimensional networks of PEG. It could be expected that PEG macromer formed a gel network and PCL chains entangled through the gel network resulting in SIPNs. The SIPNs films were mechanically strong enough to handle and became stronger with increasing PCL content due to the relative hydrophobicity of PCL.

DSC studies were shown in Table I. These results showed that the glass transition temperature of PCL in the SIPNs were inner shifted whereas pure PCL had a glass transition around −52°C. More inner shift in T_g of PCL was observed by increasing the PEG weight fraction. These results indicate the formation of the interpenetrating PCL and PEG chains or the presence of phase mixed chains.[22] Melting temperature of PCL in the SIPNs was also inner shifted as shown in Table I. But T_g and T_m of the PEG network in the SIPNs were not observed.

Water content of PCL/PEG SIPNs against an incubation time according to the weight fraction of PEG in distilled water at 37°C is shown in Figure 2. These results showed that water contents were dependent on the weight fraction of PEG in the SIPNs and the water contents increased with increasing PEG weight fraction due to the hydrophilicity of PEG. Also, the water contents rapidly increased with incorporation of PEG in the SIPNs. SIPNs composed of PCL and PEG macromer are expected to produce different degrees of matrix hydration depending on the nature, crosslinked density, and amount of PEG. As a matter of fact, the water content of PCL/PEG (10/90, w/w) (MW PEG, 20,000) was higher than that of PCL/PEG (10/90, w/w) (MW PEG, 7,500). It is probable that

Table I Thermal Properties of PEG/PCL SIPNs Measured by DSC

	T_g (°C)		T_m (°C)	
Polymer	PEG	PCL	PEG	PCL
PEG network	−14		67	
PEG/PCL (70/30)	NM	−43	NM	68
PEG/PCL (50/50)	NM	−48	NM	70
PEG/PCL (30/70)	NM	−50	NM	73
PCL		−52		75

NM, nonmeasurable.

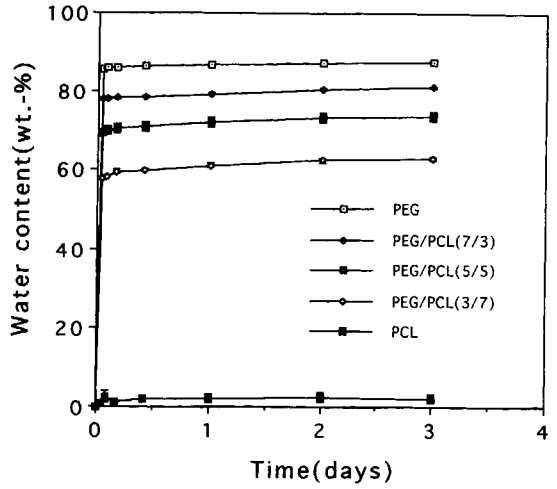

Figure 2 Water content of PCL/PEG SIPNs against weight fraction of PEG (MW of PEG, 7,500 and concn of PEG macromer, 30 wt %). Each point represents the mean ± SD of at least three experiments.

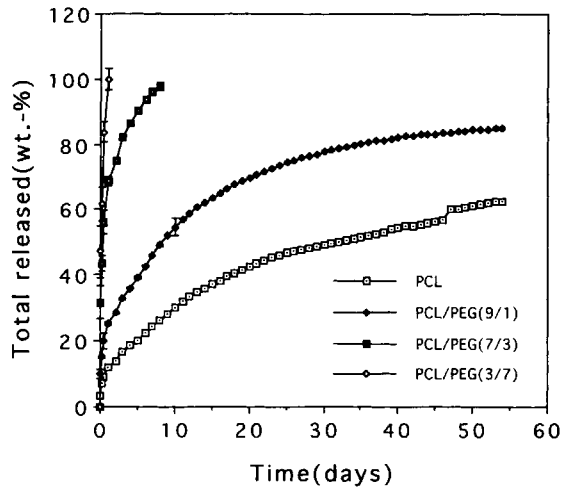

Figure 3 Released tetracycline HCl from the PCL/PEG SIPNs against weight fraction of PEG (MW of PEG: 7,500, concn of PEG macromer, 20 wt %, and drug loading, 16.7 wt %). Each point represents the mean ± SD of at least three experiments.

the higher the molecular weight of PEG macromer, the lower the crosslinking density of the PEG network and thus the higher water content. Also, the water content was dependent on the crosslinked density of PEG macromer in the SIPNs preparation. The higher the crosslinked density, the smaller the water content.

Total released of tetracycline HCl from the PCL/PEG SIPNs against weight fraction of PEG is shown in Figure 3. These results indicated that the release of tetracycline HCl from the SIPNs rapidly increased with incorporation of PEG in the SIPNs. As shown in Figure 3, the larger the amounts of PEG, the faster the release of the antibiotic drug. Almost 100% of the loaded drug was released within 1 day from the PCL/PEG (30/70, w/w) matrix whereas only 50% of the loaded drug was released within 47 days from the PCL matrix. These observed phenomena could be explained in relation to the degree of swelling of the PCL/PEG SIPNs matrices as shown in Figure 2. Therefore, the increase of the release rate was attributed to the increase of hydrophilicity of PEG in the SIPNs. Accordingly, the penetration of water molecules within SIPNs was easier and made the transport of the drug to the surrounding aqueous medium faster.

Total released of tetracycline HCl from the PCL/PEG SIPNs (70/30, w/w) against concentration of PEG macromer in the SIPNs preparation is shown in Figure 4. From these results, the lower concentration of PEG macromer leads to a faster drug release than the higher concentration of PEG macromer in the SIPNs preparation. Physical properties were improved by independently crosslinking a hydrophobic network within the crosslinked hydrophilic network.[23] This mixture of two independently crosslinked polymers that cannot be physi-

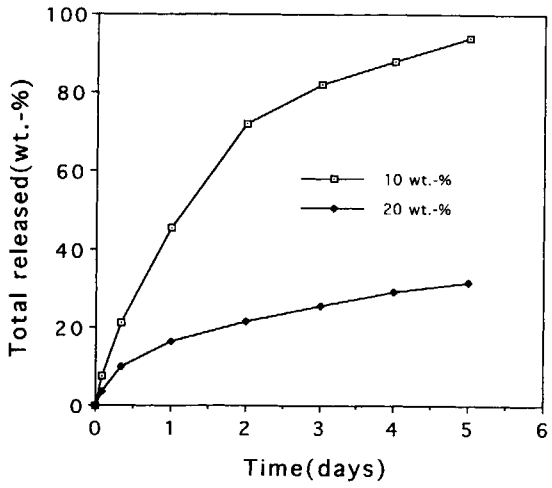

Figure 4 Released tetracycline HCl from the PCL/PEG (90/10, w/w) SIPNs against concentration of PEG macromer in the SIPNs preparation (MW of PEG, 7,500 and drug loading, 16.7 wt %). Each point represents the mean ± SD of at least three experiments.

cally separated is an IPNs. If only one of the two polymers is crosslinked, the product is called an SIPNs. It is usually made by polymerizing or crosslinking one component in the presence of the other. In this system, PEG macromer is crosslinked in the presence of PCL by UV. To make an SIPNs, two polymers should dissolve in the same solvent. An increase of concentration of the PEG macromer in the SIPNs preparation causes the increased probability that the diacrylate unit of the PEG is the site of a crosslinking bridge. Therefore, the higher concentration of PEG macromer leads to the formation of the higher crosslink density of the PEG network. Accordingly, the penetration of water molecules within SIPNs prepared by a lower concentration of PEG macromer was easier and made the drug release to the surrounding aqueous medium faster.

Total release of tetracycline HCl from the PCL/PEG SIPNs (70/30, w/w) against drug loading is shown in Figure 5. From these results, a two-phase release mechanism were observed for the two matrices. During the first phase, which lasted up to 2 days depending on the drug loading, the higher loading was released more rapidly than the lower loading. During the second phase after 2 days, total release of the drug was not much dependent on the drug loading. These results reflected occurrence of acid–base interaction of the basic drug with the chain end carboxyl groups of PCL.[12]

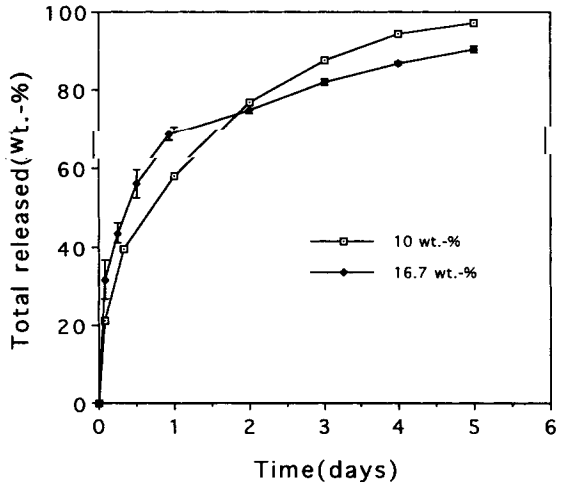

Figure 5 Released tetracycline HCl from the PCL/PEG (30/70, w/w) SIPNs against drug loading (MW of PEG, 7,500 and concn of PEG macromer, 20 wt %). Each point represents the mean ± SD of at least three experiments.

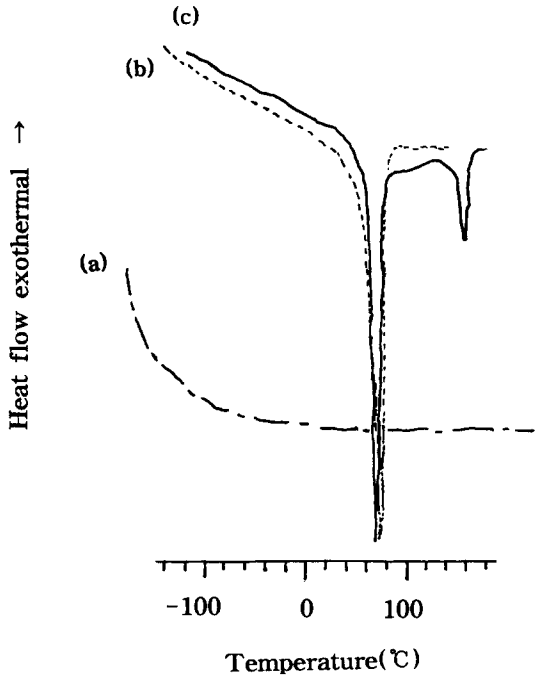

Figure 6 DSC curves for (a) tetracycline salt, (b) PCL/PEG (30/70, w/w) SIPNs, and (c) drug loaded PCL/PEG SIPNs.

Figure 6 shows DSC curves for tetracycline HCl, PCL/PEG SIPNs, and tetracycline HCl loaded PCL/PEG SIPNs. These results indicated that the T_m of PCL and decomposition temperature of the drug for the drug loaded PEG/PCL SIPNs were observed around 73 and 158°C [Fig. 6(c)], respectively, whereas the decomposition temperature of the drug itself was not found in this experiment [Fig. 6(a)]. It may be regarded that interactions between the end chain carboxylic acid groups of PCL and drug may occur, as explained in Figure 5.

Figure 7 shows SEM photographs of PCL/PEG (30/70, w/w) SIPNs before and after *in vitro* drug release. These results indicated that the morphology of the SIPNs between before and after *in vitro* drug release appeared quite different. The morphology of the SIPNs after 7 days of drug release was rather porous. This porosity accounted well for the drug release in the aqueous medium. It is likely that the porosity was formed during the drug release.

Degradation profiles for PCL/PEG SIPNs against incubation time according to the weight fraction of PEG in the SIPNs in PBS solution, pH 7.4, at 37°C are shown in Figure 8. These results showed that the degradation rate for the SIPNs decreases with increasing PCL weight fraction similar to the water content. These results are attributed

to the hydrophilic nature of PEG that increases the accessibility of water to the polymer matrix. Also, PCL has been known to degrade very slowly because of its hydrophobic structure that does not allow fast water penetration. PCL degradation by random hydrolytic chain scission of the ester linkages was documented by Pitt et al.[24] Hydrolysis events per crosslinked PEG macromer chain resulted in that polymer chain entering into solution in the aqueous surroundings.[21] From SEM photographs of the morphology of the SIPNs after 50 days *in vitro* (not shown), it can be said that degradation showed bulk bioerosion rather than surface hydrolysis. It may be expected that the degradation of the SIPNs is a function of the crosslinked density, the nature of PEG, and the mole fraction of PEG in the SIPNs.

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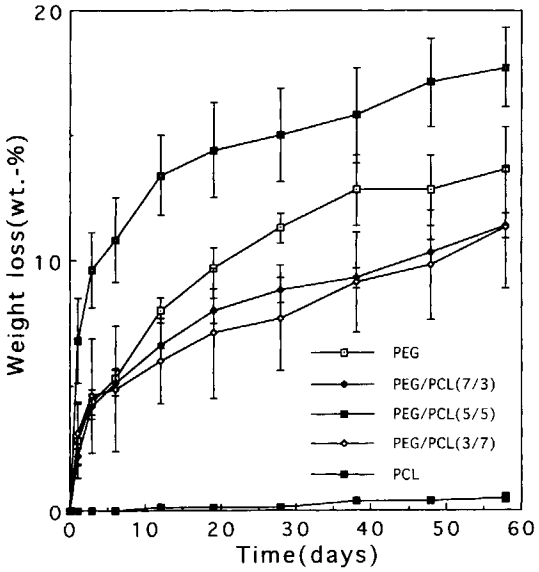

Figure 8 Degradation for the PCL/PEG SIPNs *in vitro* against weight fraction of PEG. Each point represents the mean ± SD of at least three experiments.

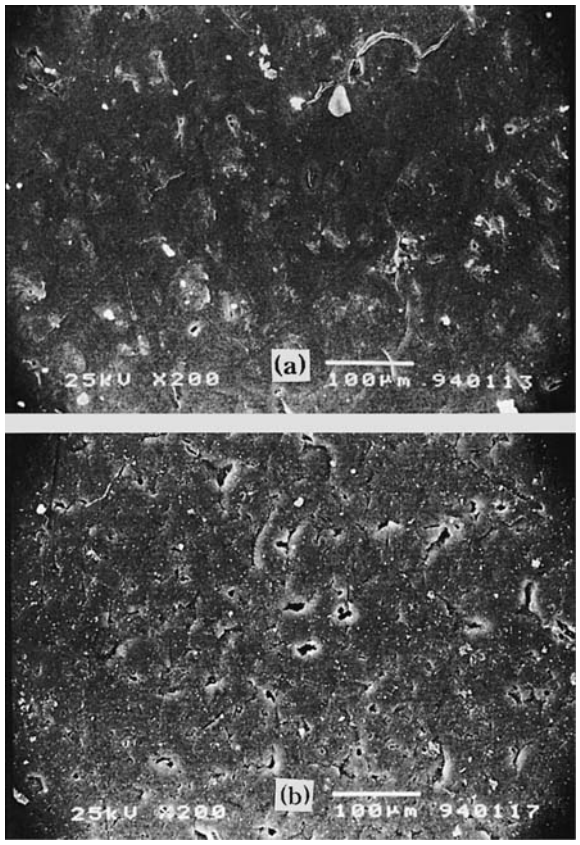

Figure 7 Scanning electron microscopy of PCL/PEG (30/70, w/w) SIPNs (a) before and (b) after *in vitro* drug release (7 days).

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