Trimethylene Carbonate and ϵ-Caprolactone Based (co)Polymer Networks: Mechanical Properties and Enzymatic Degradation

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High molecular weight trimethylene carbonate (TMC) and ϵ-caprolactone (CL) (co)polymers were synthesized. Melt pressed (co)polymer films were cross-linked by gamma irradiation (25 kGy or 50 kGy) in vacuum, yielding gel fractions of up to 70%. The effects of copolymer composition and irradiation dose on the cytotoxicity, surface properties, degradation behavior, and mechanical and thermal properties of these (co)polymers and networks were investigated. Upon incubation with cell culture medium containing extracts of (co)polymers and networks, human foreskin fibroblasts remained viable. For all (co)polymers and networks, cell viabilities were determined to be higher than 94%. The formed networks were flexible, with elastic moduli ranging from 2.7 to 5.8 MPa. Moreover, these form-stable networks were creep resistant under dynamic conditions. The permanent deformation after 2 h relaxation was as low as 1% after elongating to 50% strain for 20 times. The in vitro enzymatic erosion behavior of these hydrophobic (co)polymers and networks was investigated using aqueous lipase solutions. The erosion rates in lipase solution could be tuned linearly from 0.8 to 45 mg/(cm^2 × day) by varying the TMC to CL ratio and the irradiation dose. The copolymers and networks degraded essentially by a surface erosion mechanism.

Introduction

An important aspect of tissue engineering is the design of scaffolding materials which mimic the properties of extracellular matrix. In this respect, the physical, chemical, and biological properties and the biodegradation behavior of the materials are decisive for their suitability. In soft tissue engineering such as cardiovascular applications, scaffolds are usually subjected to mechanical stimuli, either during culturing in a bioreactor or upon implantation in the body. As the scaffold and the engineered tissue should be able to deform without dilation under cyclic pulsatile conditions, resorbable, flexible and elastic materials that allow cell adhesion and proliferation, are likely the most suited to form functional soft-tissue constructs.[1−4] In addition, surface eroding polymers might be better suited as a scaffolding material than polymers that degrade by bulk erosion, as mass loss is not preceded by significant deterioration of mechanical properties and structural integrity.[5,6]

Due to their biocompatibility and their tunable degradation rates, polymers of lactide, glycolide, and ε-caprolactone and their copolymers have initially been used as scaffolding materials.[7−11] But these polymers are relatively rigid, with elastic moduli ranging from approximately 400 MPa for poly(ϵ-caprolactone) to 3000−3500 MPa for poly(glycolide) and poly(lactide), while soft tissues, such as myocardium, have elastic moduli below 1 MPa.[12−14] At higher elongations, these polymers either fracture in a brittle manner or by plastic deformation. Furthermore, their hydrolytic degradation takes place by bulk erosion, which could lead to a rapid loss of mechanical integrity and a burst release of acidic degradation products.

High molecular weight poly(1,3-trimethylene carbonate) (PTMC) is a flexible, rubbery polymer that can be cross-linked into an elastic network upon gamma irradiation in an inert atmosphere.[15,16] This is an important feature, as networks are highly resistant to creep under long-term cyclic (pulsatile) deformation. The polymer is quite stable toward hydrolysis in buffers ranging from pH 1 to 13, whereas it degrades relatively rapidly in vivo by enzymatic surface erosion.[17−21] Several enzymes have been shown to degrade PTMC in in vitro experiments as well.[19,22,23] We compared the degradation behavior of PTMC of different molecular weights in vivo, in tibia of rabbits, and in vitro in aqueous lipase solutions (from *Thermomyces lanuginosus*).[19] In both cases, PTMC degraded by a surface erosion process, but the rate of mass loss depended on polymer molecular weight. In the lipase solutions, PTMC with a molecular weight of 29.1 × 10^4 g/mol eroded at rate of 6.7 μm/day, while PTMC of a molecular weight of 6.9 × 10^4 g/mol eroded at a rate of 1.4 μm/day. Besides this effect of polymer molecular weight on erosion rate, in vivo experiments showed that cross-linking and network formation upon gamma irradiation also decreases the rate of mass loss.[16]

Poly(ε-caprolactone) (PCL) is a semicrystalline polymer with a melting point of approximately 65 °C and a low glass transition temperature of approximately −65 °C. In a manner similar to

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PTMC, PCL can also be cross-linked to form networks by gamma and electron beam irradiation.[24−26] In vitro, several enzyme solutions have been shown to effectively degrade PCL and PCL networks.[25,27,28] Here also, the rate of mass loss was lower for networks than for linear PCL polymers. While PCL erodes within four days in *Pseudomonas lipase* (Lipase PS) solutions,[27,28] the in vivo degradation process is markedly different. Animal implantation studies have shown that it takes years for a high molecular weight PCL specimen to resorb.[29,30]

In this study, we aimed at developing flexible and elastic form-stable networks that mimic the properties of soft tissue more closely. To tune the degradation properties of TMC polymers, copolymers were prepared with CL. Furthermore, as these copolymers are expected to also cross-link upon gamma irradiation, flexible creep-resistant networks could readily be prepared in this manner. The gamma irradiation dosage and the TMC to CL ratio were varied to investigate their effects on the chemical, physical, and biological properties of TMC-CL (co)polymer networks and their in vitro enzymatic degradation behavior.

Materials and Methods

Materials. Polymer grade 1,3-trimethylene carbonate was obtained from Boehringer Ingelheim (Germany). ε-Caprolactone (Aldrich, U.K.) was dried over ground CaH_2 and purified by distillation under reduced argon atmosphere. Stannous octoate (Sigma, U.S.A.) was used as received. Lipase from *Thermomyces lanuginosus* (EC 3.1.1.3, min. 100.000 units/g) was purchased from Sigma (Denmark) and used as received. This aqueous enzyme solution further contains 25 wt % propylene glycol, 0.5 wt % $CaCl_2$, and 2 wt % enzyme concentrate. Phosphate buffered saline (PBS, pH = 7.4) was obtained from B. Braun Melsungen AG, Germany. Solvents (Merck, Germany) were of analytical grade.

Polymer Synthesis. Poly(1,3-trimethylene carbonate), poly(ε-caprolactone), and poly(1,3 trimethylene carbonate-co-ε-caprolactone) were synthesized by ring opening polymerization of the corresponding monomers under vacuum at 130 ± 2 °C for three days using stannous octoate (2×10^{-4} mol per mol of monomer) as catalyst. The polymers were purified by dissolution in chloroform and precipitation into a 10-fold volume of ethanol, washing with ethanol, and drying at room temperature under vacuum.

Polymer Characterization. Monomer conversion and copolymer composition were determined by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy (300 MHz, Varian Innova, USA) using $CDCl_3$ (Merck, Germany).

Glass transition temperatures (T_g) and melting temperatures of purified polymers and irradiated polymer films were determined by differential scanning calorimetry (DSC). Samples (5−10 mg) were analyzed at a heating rate of 10 °C/min in a temperature range of −100 to 100 °C using a Perkin-Elmer Pyris 1 DSC. After the first scan, samples were quenched to −100 at 300 °C/min and a second scan was recorded after 5 min. Reported values were determined from the second scan. Indium, lead, and cyclohexane were used as standards for temperature calibration.

Number average and weight average molecular weights ($\bar{M}_n$ and $\bar{M}w$, respectively), molecular weight distributions (MWD), and intrinsic viscosities ([η]) of the purified polymers and extracts of irradiated polymers were determined by gel permeation chromatography (GPC, Viscotek, U.S.A.). The setup was equipped with ViscoGEL I-guard-0478, ViscoGEL I-MBHMW-3078, and ViscoGEL I-MBLMW-3078 columns placed in series and a TDA 302 Triple Detector Array with refractometer, viscometer, and light scattering detectors, allowing the determination of absolute molecular weights. All determinations were performed at 30 °C, using chloroform as the eluent at a flow rate of 1.0 mL/min.

Polymer Processing. Purified polymers were compression molded at 140 °C in 500 μm thick stainless steel molds using a laboratory press (Fonteijne THB008, The Netherlands). The films were molded at approximately 25 kg/cm^2 and quenched to room temperature using cold water.

Gamma Irradiation, Network Formation, and Network Characterization. The compression molded films were sealed under vacuum in laminated polyethylene/polyamide bags (Hevel Vacuum B.V., The Netherlands) and exposed to 25 or 50 kGy gamma irradiation from a ^{60}Co source (Isotron B.V., Ede, The Netherlands). This leads to cross-linking and network formation.[15,16]

To determine equilibrium swelling and gel contents, disk-shaped specimens (500 μm thick, 10 mm in diameter) were punched out from the irradiated films and placed in 30 mL of $CHCl_3$ for 1 week, the solvent was refreshed once after 3 days. This procedure ensured complete removal of the sol fraction. Then the swollen gels were weighed, dried to constant weight at room temperature in vacuo, and weighed again. The gel and the sol fractions were calculated according to eqs 1 and 2, respectively

$$\text{gel fraction}(\%) = \frac{m_d}{m_0} \times 100 \quad (1)$$

$$\text{sol fraction}(\%) = \left(1 - \frac{m_d}{m_0}\right) \times 100 \quad (2)$$

where m_d is the mass of dried (extracted) samples and m_0 is the mass of the specimens before swelling. The volume degree of swelling (q) was calculated according to eq 3.

$$q = 1 + \rho_p \times \left(\frac{m_s}{m_d \times \rho_s} - \frac{1}{\rho_s}\right) \quad (3)$$

where m_s is the mass of the extracted and swollen samples and ρ_s and ρ_p are the densities of chloroform[31] (1.4832 g/cm^3) and the (co)polymers, respectively. The densities of the copolymers were determined by measuring the mass and dimensions of compression molded films. The densities of TMC and CL copolymers containing 100, 89, 79, 70, and 0% TMC were 1.31, 1.29, 1.27, 1.25, and 1.09 g/cm^3, respectively.

Cell Viability Assay. Possible cytotoxicity of the polymer films irradiated at 0, 25, or 50 kGy was evaluated using a [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium] (MTS) assay.[32] MTS is a tetrazolium compound that is reduced by living cells into a formazan product, the absorbance of which can be measured at 490 nm. The quantity of formazan produced is directly proportional to the number of living cells in culture.

Human skin fibroblasts (PK 84 cell line) were cultured in a 96-well plate (5000 cells/well) in RPMI medium (Roswell Park Memorial Institute medium). Of each sample, two disks (diameter 6 mm, thickness 500 μm) were extracted at 37 °C and 5% CO_2 with 400 μL RPMI for 24 h. The cells were incubated at 37 °C and 5% CO_2 for three days, the medium was then replaced by RPMI medium containing the polymer extract. After two days, the absorbance of formazan, which is soluble in the culture medium, was measured. The mean value obtained for cell cultures incubated with RPMI medium only was standardized as 100% cell viability.

A medical grade polyurethane (2363-55D-Pellethane resin from Dow Chemical, Midland, U.S.A.) and latex rubber (Hilversum Rubber Factory, Hilversum, The Netherlands) were used as negative and positive controls, respectively. Prior to the MTS assays, it was confirmed that the controls and the (co)polymer samples were essentially endotoxin-free.

Mechanical Properties. The mechanical properties of melt pressed and irradiated (0 kGy, 25 kGy, and 50 kGy) (co)polymers were determined in triplicate according to ASTM-D 882-91. The specimens were not extracted after irradiation and measured 0.5 × 10 × 0.05 cm^3. A Zwick Z020 tensile tester (Ulm, Germany) equipped with a 500 N load cell was operated at a crosshead speed of 500 mm/min.

Table 1. Characteristics of Synthesized TMC-CL (co)Polymers Before and After Compression Moulding[a]

TMC/CL charged (mol ratio)	TMC/CL[b] actual (mol ratio)	T_g[c] (°C)	$\bar{M}_n$[d] (kg/mol)	MWD[d]	$[\eta]$[d] (dl/g)
100/0	100/0	−14.8	590 (586)	1.31 (1.32)	7.5 (7.2)
90/10	88.9/11.1	−23.4	149 (136)	1.60 (1.62)	2.8 (2.6)
80/20	79.2/20.8	−29.2	264 (218)	1.39 (1.56)	4.3 (4.0)
70/30	69.9/30.1	−35.8	261 (230)	1.35 (1.43)	4.2 (4.1)
0/100	0/100	−64.3	234 (228)	1.35 (1.37)	4.7 (4.6)

[a] The values for compression molded specimens are given between brackets. [b] Determined by ^{1}H NMR on purified specimens. [c] Determined by DSC from the second scan. [d] Determined by GPC at 30 °C using chloroform as the eluent.

The initial grip to grip separation was 50 mm and a preload of 0.01 N was applied. The strain was measured using extensometers.

To assess their behavior under dynamic loading conditions, the specimens were repetitively (20×) elongated to 50% strain at 50 mm/min in cyclic tests. After a 2 h recovery period, the permanent deformation was estimated from the stress−strain diagram of the 21st cycle. In these experiments a preload of 0.01 N was applied, and the deformation was derived from the grip to grip separation. The error in the values is approximately 0.5% strain.

Wettability and Water Uptake. Captive bubble contact angles were determined using ultrapure water (MilliQ Plus-Millipore, France) at room temperature and a video-based system (OCA 15 DataPhysics Instruments GmbH, Germany). Glass discs ($n = 3$), were spin-coated with (co)polymer solutions in chloroform (2−3 wt/vol %), dried in vacuum, and cross-linked by gamma irradiation as described before. Measurements (three per disk) were performed immediately after immersion in the water and after conditioning for a week.

The equilibrium water uptake of disks (500 μm thick, 10 mm in diameter) was determined after conditioning at 37 °C in PBS (pH = 7.4) for 1 week. Water uptake was defined as the mass increase of the disks.

In Vitro Enzymatic Degradation. The in vitro enzymatic degradation of TMC-CL (co)polymers and networks was investigated using aqueous lipase solutions (lipase from *Thermomyces lanuginosus*, min. 100.000 units/g). Nonextracted, disk-shaped specimens (500 μm thick, 10 mm in diameter; $n = 3$/time point) were placed in vials containing 0.5 mL of the enzyme solution and conditioned at 37 °C. The medium was refreshed twice a week, except for PCL polymers and networks, where the medium was refreshed every 8 h. Control experiments without enzyme were performed using PBS (pH 7.4; $n = 1$ per time point). At predetermined times, the mass and thickness of wet specimens was determined after blotting the surface. The same measurements were then performed after rinsing, and drying the specimens to constant weight in vacuo at room temperature.

Results and Discussion

To prepare flexible and form-stable (co)polymer networks by gamma irradiation, a range of (co)polymers based on TMC and CL were synthesized. The TMC to CL ratio and the gamma-irradiation dose were varied to obtain networks with tunable properties.

Synthesis of (co)Polymers. High molecular weight (co)polymers were synthesized by ring opening polymerization of the corresponding monomers. In all cases, the monomer conversion was quite high (>98%). Table 1 gives the characteristics of the purified and of the compression molded (co)polymers.

The molecular weights of the (co)polymers were relatively high (149 to 590 × 10^3 g/mol), although the copolymer containing 89 mol % TMC had a somewhat lower molecular weight than the other polymers. The comonomer ratios determined for the purified copolymers were in accordance with the amounts charged. The glass transition temperatures (T_g) of all (co)polymers were below 37 °C. PTMC and TMC-CL copolymers were amorphous and, therefore, in the rubbery state at physiological temperatures. Only PCL was semicrystalline, with a maximum melting temperature of 55.6 °C and a heat of fusion of 53.4 J/g.

Gamma Irradiation and Network Formation. All polymers cross-linked upon gamma irradiation. The networks were characterized by performing equilibrium swelling tests using chloroform as the solvent. Gel fractions and degrees of swelling of the networks are given in Table 2. Especially at the higher irradiation doses, networks with gel fractions up to 70% could be prepared. These values are comparable to those of networks prepared from high molecular weight PTMC.[15,16]

Table 2 shows that for all (co)polymers the gel fraction of the networks increases, and the degree of swelling decreases, as the irradiation dose is increased from 25 to 50 kGy. In assessing the effect of the copolymer composition on the network characteristics, no major differences in swelling behavior can be observed at irradiation doses of 50 kGy. However, at 25 kGy networks with significantly lower gel contents are obtained from the copolymers. This may be due to the relatively low initial molecular weights of the copolymers as compared to PTMC[15,16] (See Table 1). Also in the case of PCL networks, the gel fractions are much higher than those previously reported.[25] Although the molecular weight of this polymer is also relatively low ($\bar{M}_n = 248$ kg/mol), it is significantly higher than that used in the earlier experiments ($\bar{M}_n = 128$ kg/mol). Apparently, in the case of cross-linking of semicrystalline PCL by gamma irradiation, higher gel fractions can be obtained at lower molecular weights when compared to amorphous PTMC.

Gamma irradiated (co)polymer films were extracted using chloroform, the molecular weights of the sol fractions are also given in Table 2. The sol fractions had lower $\bar{M}_n$ values and a broader MWD compared to the corresponding nonirradiated (co)polymers. As the irradiation dose was increased, the $\bar{M}_n$ values of the sol fractions decreased, while the molecular weight distribution became broader. This indicates that upon gamma irradiation, degradation, branching, and cross-linking occur simultaneously. The exact reaction mechanisms of the chain scission and cross-linking processes involved are not known.

High energy irradiation can lead to radical formation due to excitation of the polymer molecules. In the case of polyethylene,[33] radicals can be formed by breakage of (1) a C−C bond in the main chain or (2) by breakage of a C−H bond, and the latter reaction will lead to simultaneous formation of a radical on the polymer chain and a hydrogen radical. In the case of TMC-CL (co)polymers, a C−O bond in the main chain can be broken as well (3). These different radicals can react with each other and with the polymer chains: Recombination of type (1) or type (3) radicals will result in the formation of either C−C bonds or C−O−C bonds, both leading to linear chains. Combination of type (2) radicals will result in cross-linking, while combination of a type (2) radical with either a type (1) or type (3) radical will yield branched structures. Branched structures also form by reaction of types (1) and (3) radicals with the linear chain. Chain scission can happen as a result of the combination of a hydrogen radical with a type (1) or type (3) polymer chain radical. Furthermore, a hydrogen radical can abstract a hydrogen atom from the main chain to form hydrogen gas and a type (2) or type (3) polymer chain radical or a double bond in the main chain. We have observed the formation of gas bubbles within PTMC films, especially at relatively low

Table 2. Effect of (co)Polymer Composition and Irradiation Dose on Network Characteristics of Formed TMC-CL (co)Polymer Networks[a]

		TMC content (mol %)				
	irradiation dose (kGy)	100	89	79	70	0
gel fraction (%)	25	57 ± 9	32 ± 3	32 ± 2	27 ± 9	57 ± 2
	50	70 ± 3	62 ± 1	63 ± 1	70 ± 11	69 ± 1
degree of swelling[b]	25	58 ± 24	163 ± 17	163 ± 15	177 ± 17	55 ± 5
	50	26 ± 4	36 ± 2	35 ± 1	33 ± 6	28 ± 9
$\bar{M}$n (sol fraction)[c] (kg/mol)	25	45.9	71.7	79.3	113.5	33.7
	50	21.8	18.7	15.5	16.5	13.9
MWD (sol fraction)[c]	25	2.64	3.23	2.99	3.15	3.35
	50	3.27	4.50	5.23	6.85	3.75

[a] Values are expressed as mean ± standard deviation ($n = 3$). [b] Determined by using chloroform at room temperature. [c] Determined by GPC at 30 °C using chloroform as the eluent.

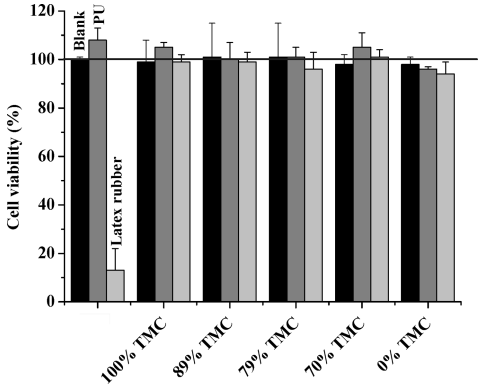

Figure 1. Cytotoxicity scores of TMC-CL (co)polymers and networks: (black) 0 kGy, (dark gray) 25 kGy, and (light gray) 50 kGy. The first three bars indicate the results of the blank (culture medium only), negative, and positive controls, respectively.

polymer molecular weights and at higher irradiation doses. This can indicate the formation of hydrogen gas, although it can not be excluded that CO or CO_2 are formed. The elucidation of the mechanism of the scissioning and cross-linking reactions will require more detailed studies.

Cell Viability. An MTS assay was used to assess the effect of extracts of (co)polymer and network films on the viability of human foreskin fibroblasts. The (co)polymers and networks were extracted using RPMI medium. The metabolic activity of the fibroblasts was determined after incubating with the medium containing the polymer extracts. A medical grade polyurethane and latex rubber were used as negative and positive control, respectively. The mean value obtained for cell cultures incubated in medium that did not contain polymer extract was standardized as 100% cell viability (blank). Figure 1 shows the results of the MTS assays.

The results of the MTS assays showed that the viability of human skin fibroblasts after incubation with extracts of the TMC and CL (co)polymer networks was quite high (>94%). All (co)polymers can be considered noncytotoxic, regardless of their monomer composition and the irradiation dose used in network formation.

Thermal and Mechanical Properties. The effects of comonomer ratio and gamma irradiation on the thermal and mechanical properties of the (co)polymers and networks are shown in Table 3.

The (co)polymers and networks containing 100, 89, 79, and 70% TMC were rubbery at physiological temperatures, with T_gs ranging from −14.8 to −36.0 °C. With decreasing TMC content, T_gs decreased. The T_gs decreased very slightly with increasing irradiation dose. PCL and PCL networks were semicrystalline at physiological temperatures. The maximum melting temperatures were 55.6, 55.7, and 55.9 °C with heats of fusion of 53.4, 56.9, 56.5 J/g for specimens treated with 0, 25, or 50 kGy gamma-irradiation, respectively. The glass transition temperatures were approximately −64 °C.

For PTMC, the elastic modulus, yield strength, yield strain, and tensile strength decreased with increasing irradiation dose, while the elongation at break increased. Relatively low molecular weight chains formed by chain scissioning upon gamma irradiation may have a plasticizing effect which will decrease the elastic modulus, yield strength, and strain. The relatively higher tensile strength of the noncross-linked samples can be related to strain induced crystallization.[15] The presence of cross-links lowers the crystallinity that can be attained upon elongation of PTMC.

The amorphous copolymers and networks containing 89, 79, and 70% TMC were also very flexible with elastic moduli ranging between 2.7−4.6 MPa. The elastic moduli and the yield strengths of the copolymers and copolymer networks decreased with decreasing TMC content and with increasing irradiation dose. Upon gamma irradiation, yielding occurred at higher strains. Gamma irradiation also dramatically increased the maximum elongation of these copolymers; films of these copolymer networks did not break at strains exceeding approximately 1000% (at this point, the limits of the extensometers were reached). The maximum stress reached for unirradiated copolymers is comparable to that of the copolymers irradiated at 25 kGy. For copolymers irradiated at 50 kGy, these values were much higher, in which the stress−strain curves showed an upturn at high strains (>800%). This could be due to denser network formation at 50 kGy compared to 25 kGy, which could lead a to more limited extensibility of the networks.

The effect of gamma irradiation on the properties of PCL was different than that on the copolymers and on PTMC. The elastic modulus and yield strength of PCL increased as the irradiation dose was increased. When PCL is irradiated at room temperature, the crystallinity is retained and cross-links are formed in the amorphous parts, leading to the higher moduli values at higher irradiation doses. However, the maximum tensile strength of PCL decreased with increasing irradiation dose. This can be related to hindered strain-induced crystallization due to the presence of cross-links.

Creep resistance is important when materials are used under dynamic loading conditions such as in cardiovascular tissue engineering. To assess this, the (co)polymers and networks were cyclically stretched to 50% strain (20 times). After two hours of recovery, a value for the permanent set was estimated from the 21st cycle (Table 3).

Before gamma irradiation, the permanent deformation of copolymers containing 79 and 89% TMC were relatively high (above 11%). The 30% CL containing copolymer even broke

Table 3. Effect of Copolymer Composition and Irradiation Dose on Glass Transition Temperature and Mechanical Properties of TMC-CL (co)Polymers and Networks[a]

TMC content (mol %)	irradiation dose (kGy)	T_g (°C)	E (MPa)	σ_{yield} (MPa)	ε_{yield} (%)	σ_{max} (MPa)	ε_{break} (%)	permanent set (%)[b]
100	0	−14.8	7.2 ± 0.4	3.2 ± 0.1	183 ± 15	15.9 ± 4.2	469 ± 26	1.3
100	25	−15.6	5.8 ± 0.1	2.4 ± 0.1	150 ± 9	9.8 ± 1.0	628 ± 2	1.0
100	50	−15.5	4.8 ± 0.3	1.9 ± 0.1[c]	136 ± 3[c]	5.9 ± 0.6	708 ± 6	1.2
89	0	−23.4	4.1 ± 0.1	1.5 ± 0.1	107 ± 5	1.5 ± 0.1	284 ± 109	11.9
89	25	−23.4	4.2 ± 0.2	1.6 ± 0.1	141 ± 8	≥1.9 ± 0.3	≥1200	1.8
89	50	−23.5	3.3 ± 0.1	1.4 ± 0.1[c]	125 ± 5[c]	≥6.6 ± 0.4	≥985	1.3
79	0	−29.2	4.6 ± 0.1	1.7 ± 0.1	95 ± 3	1.7 ± 0.1	103 ± 6	11.0
79	25	−30.1	3.8 ± 0.1	1.4 ± 0.1	133 ± 4	≥1.6 ± 0.1	≥1230	1.2
79	50	−29.8	3.1 ± 0.1	1.3 ± 0.1[c]	126 ± 5[c]	≥7.1 ± 0.4	≥1010	1.4
70	0	−35.8	4.2 ± 0.2	1.5 ± 0.1	84 ± 2	1.5 ± 0.1	91 ± 5	[d]
70	25	−35.7	3.3 ± 0.1	1.2 ± 0.1	137 ± 3	≥1.3 ± 0.1	≥1235	1.4
70	50	−36.0	2.7 ± 0.1	1.1 ± 0.1[c]	125 ± 5[c]	≥4.9 ± 0.5	≥973	1.4
0	0	−64.3	458 ± 18	17.1 ± 0.2	11 ± 2	38.3 ± 2.1	442 ± 2	34.3
0	25	−64.2	493 ± 21	18.3 ± 0.1	12 ± 1	35.7 ± 2.0	467 ± 12	35.0
0	50	−64.3	514 ± 5	19.0 ± 0.3	13 ± 1	30.9 ± 1.7	414 ± 6	36.4

[a] Values are expressed as mean ± standard deviation ($n = 3$). [b] Result of a single measurement. Permanent set is estimated from the 21st cycle performed after two hours of recovery period. The error is approximately 0.5% strain. [c] Estimated from the intersection of tangents to stress−strain diagrams as the curves did not show a distinct yield point. [d] Sample broke during the cyclic loading experiment.

during the experiment. Upon gamma irradiation and cross-linking, the creep resistance of all copolymer networks was excellent, with a permanent deformation below 2%. The irradiation dose itself had only a very small effect on these values. Remarkably, PTMC also had a very low permanent deformation (1.3%) in the noncross-linked state; this can probably be related to its very high molecular weight. Here, gamma irradiation only slightly reduced permanent deformation.

Under the same dynamic loading conditions, PCL and PCL networks had poor resistance to creep, with permanent deformations at room temperature and at 37 °C, greater than 34%. This is a result of their semicrystalline structure. Upon heating to 70 °C, which is above the melting temperature, the films returned to their original dimensions within seconds.

Contact Angles and Water Uptake. Captive bubble contact angles of the (co)polymer and network films on glass discs were determined immediately after immersion in ultrapure water and after conditioning for one week. Before irradiation the (co)-polymers were hydrophobic, having contact angles between 58 and 65° as measured immediately after immersion in water. Upon gamma irradiation, the surfaces become more hydrophobic, as higher contact angles (63−70°) were determined. No significant effect of the polymer composition could be observed. After a one-week conditioning in water, the contact angles of all films decreased. The values ranged between 52 and 55° for nonirradiated films and between 52 and 61° for gamma-irradiated films. This is in line with the uptake of small amounts of water, allowing some reorientation or diffusion of polymer end groups at the polymer−water interface.

All the (co)polymers took up less than 2 wt % water. The water uptake of PCL was the lowest; which could be related to its chemical structure and crystallinity. TMC content and irradiation dose did not have a large influence on contact angles and water uptake.

In Vitro Enzymatic Degradation. The in vitro enzymatic degradation of melt pressed films irradiated at 0, 25, or 50 kGy was investigated using aqueous solutions of lipase from *Thermomyces lanuginosus*. All (co)polymers and networks eroded in time when incubated in the lipase solutions at 37 °C. During degradation, the films preserved their integrity and the enzyme solutions remained clear. No significant mass loss was observed in control experiments, where only PBS (pH = 7.4) was used. This implies that the contribution of nonenzymatic hydrolysis to the degradation process was minimal.

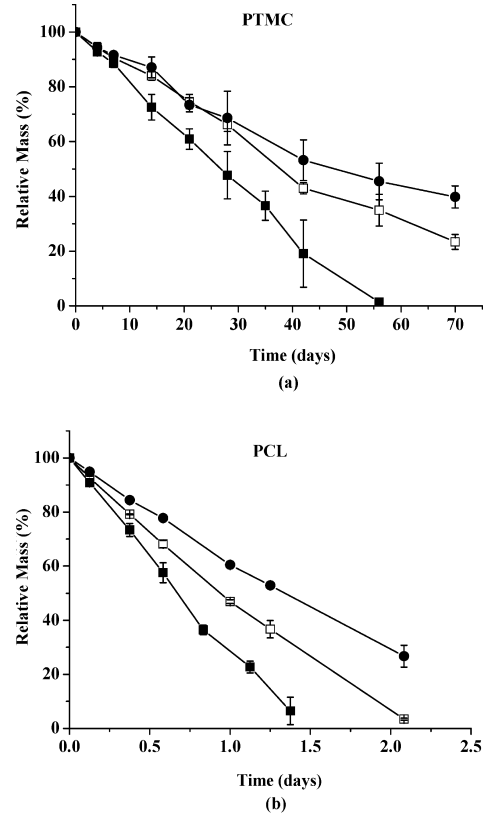

Figure 2. Effect of irradiation dose on the erosion behavior of PTMC and PCL homopolymers in lipase solutions: (a) PTMC, (b) PCL; (■) 0, (□) 25, (●) 50 kGy. Initially, the PTMC disks measured 10 mm in diameter and approximately 530 μm in thickness, weighing approximately 57 mg. PCL disks measured 10 mm in diameter and approximately 580 μm in thickness, weighing approximately 51 mg.

Figure 2 shows the change in relative mass of PTMC and PCL specimens irradiated at 0, 25, or 50 kGy in time. The complete erosion of nonirradiated PTMC took approximately eight weeks, whereas at the same time point the relative mass loss for PTMC irradiated at 50 kGy was only about 55%. Nonirradiated PCL lost more than 90% of its mass after 33 h, while PCL cross-linked at 50 kGy lost approximately 75% of its mass after 50 h.

Table 4. Effect of Copolymer Composition and Irradiation Dose on the Erosion Rate of TMC-CL (co)Polymers and Networks in Lipase Solutions[a]

	0 kGy		25 kGy		50 kGy	
	erosion rate[b]		erosion rate[b]		erosion rate[b]	
dose, TMC %	mg/(cm^2 × day)	μm/day	mg/(cm^2 × day)	μm/day	mg/(cm^2 × day)	μm/day
100	1.3 ± 0.1	[c]	0.9 ± 0.1	6.0 ± 0.2	0.8 ± 0.1	4.6 ± 0.4
89	1.3 ± 0.1	[c]	2.0 ± 0.1	13.8 ± 0.2	2.2 ± 0.1	13.3 ± 0.3
79	5.1 ± 0.5	[c]	4.0 ± 0.1	28.3 ± 2.3	4.1 ± 0.3	29.6 ± 2.5
70	9.7 ± 1.3	[c]	8.0 ± 0.5	60.0 ± 6.2	9.0 ± 0.3	57.9 ± 2.7
0	45.0 ± 1.4	301 ± 13	32.5 ± 1.2	222 ± 3	24.8 ± 0.6	160 ± 4

[a] Disks with initial diameters of 10 mm and thicknesses of 500−590 μm were incubated. Initial masses ranged from 51 to 57 mg. [b] Values are calculated from the initial linear region of the curves ($R > 0.99$). [c] No reliable data could be obtained due to poor form stability of the specimens.

The erosion rates of (co)polymers and networks calculated from this mass loss data are given in Table 4. For PTMC, the rate of mass loss decreased from 1.3 to 0.8 mg/(cm^2 × day) when the irradiation dose was increased from 0 to 50 kGy. For PCL, the rates decreased from 45.0 to 24.8 mg/(cm^2 × day) after increasing the irradiation dose from 0 to 50 kGy. The decrease in degradation rate with increasing irradiation dose may be due to hindered chain mobility as a result of (denser) network formation. Although the use of a lipase solution was previously shown to be a good model for the in vivo erosion of PTMC,[19] the erosion rate of PCL is very much higher when exposed to aqueous lipase solutions than that in vivo.[29,30]

Macrophages are usually the dominant type of cells seen at the interface between the tissue and the degradable polymer. These cells can secrete many different enzymes, such as esterases and lipases, that could degrade these TMC (co)polymers and networks. However, exactly which enzymes are responsible for the in vivo erosion of these (co)polymers and networks is not known.

The erosion behavior of TMC and CL (co)polymers irradiated at 0, 25, or 50 kGy is illustrated in Figure 3. The copolymer composition had a significant effect on the rate of degradation. Upon increasing the TMC content of the polymers and networks, erosion rates decreased. At 0 kGy, the erosion rates of the (co)polymers decreased linearly ($R = 0.98$) from 45.0 mg/(cm^2 × day) to 1.3 mg/(cm^2 × day) as the TMC content of the (co)polymer was decreased from 100 to 0%. See also the data presented in Table 4. When comparing the behavior of the polymers (Figure 3), nonirradiated (0 kGy) 89% TMC-containing copolymer eroded at a relatively low rate. This could be due to its relatively low molecular weight.[18]

After irradiation and network formation, the same linear trend was observed. At an irradiation dose of 25 kGy and 50 kGy, the erosion rates of the (co)polymer networks linearly ($R = 0.99$ for both 25 and 50 kGy) decreased from 32.5 to 0.9 mg/(cm^2 × day) and from 24.8 to 0.8 mg/(cm^2 × day), respectively. The erosion rates of the copolymer networks were not much influenced by irradiation dose, which is different than in the case of PTMC and PCL networks. The effect of copolymer composition on the erosion rates was stronger than the effect of irradiation dose.

The linear increase in erosion rates with the decrease in TMC content suggests that the catalytic activity of the enzyme in cleaving ester bonds is higher than in cleaving carbonate bonds. ^{1}H NMR measurements have shown that the TMC content in the copolymers increases slightly as the films degrade. For example, the TMC content of a nonirradiated specimen, that inititally contained 79% TMC, had increased to 84% after a one week degradation in lipase solution (mass loss = 56%).

It has been shown that the activity of lipases is enhanced at the water−lipid interface and when adsorbed on hydrophobic supports.[34] This is due to a conformational change, after which the active site of the enzyme, normally buried beneath a helical segment called "lid", becomes accessible. The active site of the enzyme contains an Asp-His-Ser triad, which is chemically analogous to serine proteases.[35] Deckwer et al. have shown that the mobility of the polymer chain is an important factor that

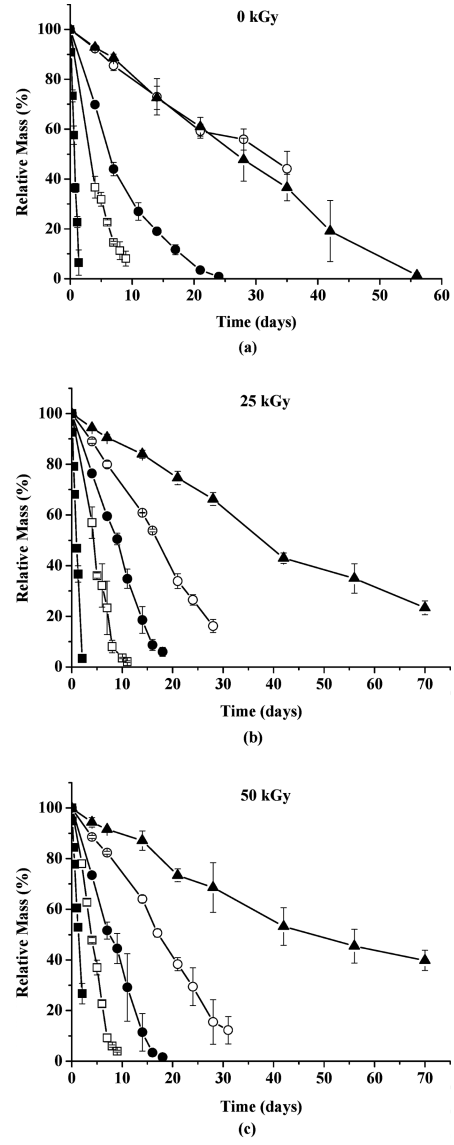

Figure 3. Effect of copolymer composition on the erosion behavior of TMC-CL copolymers and networks: (a) 0, (b) 25, (c) 50 kGy; (▲) 100% TMC, (○) 89% TMC, (●) 79% TMC, (□) 70% TMC, (■) 0% TMC (disks with initial diameters of 10 mm and thicknesses of 500−590 μm were incubated. Initial masses ranged from 51 to 57 mg).

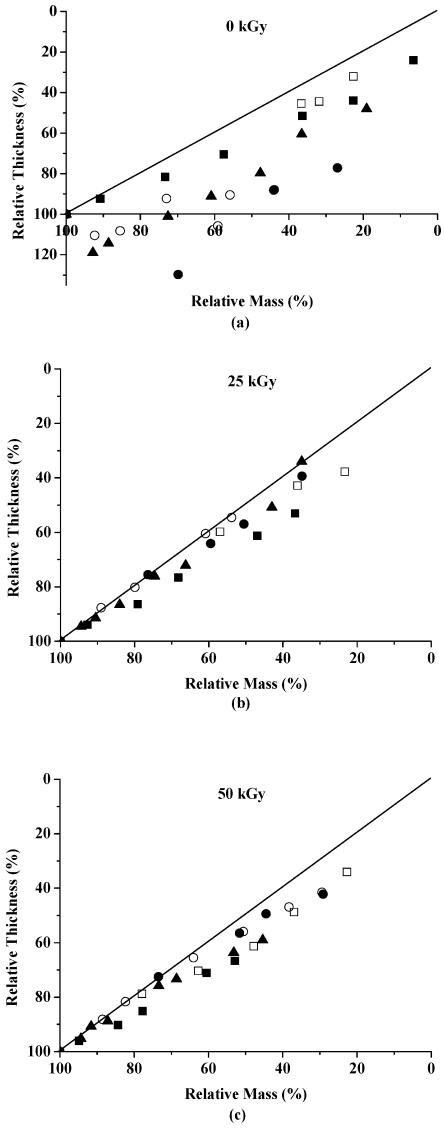

Figure 4. Relationship between the loss in relative thickness and relative mass of TMC-CL copolymers and networks during degradation in lipase solutions: (a) 0, (b) 25, (c) 50 kGy; (▲) 100% TMC, (○) 89% TMC, (●) 79% TMC, (□) 70% TMC, (■) 0% TMC.

controls the degradability of polyesters.[36] The decrease in T_gs (See Table 3) of the (co)polymers and networks with an increase in CL content could have an enhancing effect on the polymer chain mobility, which would make the chains more accessible to the active site of the enzyme. Despite its semicrystalline structure, PCL, which has a glass transition temperature of −64 °C, had degraded completely and at a much faster rate than the other (co)polymers. This suggests that both amorphous and crystalline domains of PCL could be degraded in lipase solutions.[27,28] In the case of PCL, apparently, the effect of crystallinity was less than that of chain mobility at 37 °C.

The changes in relative thickness of the films during degradation in lipase solutions and the relationship between the change in relative mass and the relative thickness are shown in Figure 4. For gamma irradiated samples, the decrease in thickness is in quite good agreement with the decrease in mass, indicating that these form-stable copolymer networks are degrading by surface erosion. Upon gamma irradiation and cross-linking, the form stability of the disks in aqueous lipase solutions at 37 °C was good. The diameter of the cross-linked specimens remained essentially unchanged during degradation. For nonirradiated specimens (0 kGy), the correlation between relative thickness and mass is present, but less specific, and as in amorphous films, the disk-shape was not preserved. This is most likely due to deformation due to creep of these low T_g (co)polymers. This low form stability was most pronounced for the 89% TMC containing copolymer, which has a relatively low molecular weight. A similar behavior was reported for low molecular weight PTMC.[19,21]

The thickness of the specimens decreased more rapidly as the CL content of the copolymers and networks was increased. By varying the TMC/CL ratio from 100:0 to 0:100, the erosion rates (decrease of thickness in time) of the networks could be tuned linearly from 6.0 to 222 μm/day for specimens irradiated at 25 kGy ($R = 0.99$) and from 4.6 to 160 μm/day for specimens irradiated at 50 kGy ($R = 0.99$; see Table 4).

Increasing the irradiation dose had a larger effect on the erosion rates of PTMC and PCL homopolymer networks than of copolymer networks. As the irradiation dose was increased to 50 kGy, the erosion rate (Table 4) decreased from 6.0 to 4.6 μm/day for PTMC materials and from 301 to 160 μm/day for PCL materials.

Conclusions

High molecular weight (co)polymers of TMC and CL were synthesized by ring opening polymerization. Flexible, form-stable, and elastic networks were obtained upon gamma irradiation. These hydrophobic polymers and networks were noncytotoxic for all the compositions and irradiation doses investigated. The (co)polymers and networks degraded in aquous lipase solutions by a surface erosion mechanism. The mechanical properties and the in vitro enzymatic erosion behavior of the copolymers and networks could readily be tuned by adjusting both the gamma irradiation dose and the TMC to CL ratio.

These flexible and elastic surface eroding networks are well-suited for use in soft tissue engineering scaffolds. Future studies will focus on the tissue response to these networks upon implantation and on their in vivo degradation behavior.

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References and Notes

(1) Zammaretti, P.; Jaconi, M. *Curr. Opin. Biotechnol.* **2004**, *15* (5), 430–434.
(2) Zimmermann, W. H.; Eschenhagen, T. *Heart Failure Rev.* **2003**, *8* (3), 259–269.
(3) Shachar, M.; Cohen, S. *Heart Failure Rev.* **2003**, *8* (3), 271–276.
(4) Yang, S. F.; Leong, K. F.; Du, Z. H.; Chua, C. K. *Tissue Eng.* **2001**, *7* (6), 679–689.
(5) Andriano, K. P.; Tabata, Y.; Ikada, Y.; Heller, J. *J. Biomed. Mater. Res.* **1999**, *48* (5), 602–612.
(6) Gopferich, A. *Biomaterials* **1996**, *17* (2), 103–114.
(7) Carrier, R. L.; Papadaki, M.; Rupnick, M.; Schoen, F. J.; Bursac, N.; Langer, R.; Freed, L. E.; Vunjak-Novakovic, G. *Biotechnol. Bioeng.* **1999**, *64* (5), 580–589.
(8) Bursac, N.; Papadaki, M.; Cohen, R. J.; Schoen, F. J.; Eisenberg, S. R.; Carrier, R.; Vunjak-Novakovic, G.; Freed, L. E. *Am. J. Physiol.* **1999**, *277* (2), H433–H444.
(9) Papadaki, M.; Bursac, N.; Langer, R.; Merok, J.; Vunjak-Novakovic, G.; Freed, L. E. *Am. J. Physiol.* **2001**, *280* (1), H168–H178.
(10) Park, H.; Radisic, M.; Lim, J. O.; Chang, B. H.; Vunjak-Novakovic, G. *In Vitro Cell. Dev. Biol.: Anim.* **2005**, *41* (7), 188–196.
(11) Ozawa, T.; Mickle, D. A. G.; Weisel, R. D.; Matsubayashi, K.; Fujii, T.; Fedak, P. W. M.; Koyama, N.; Ikada, Y.; Li, R. K. *Cell Transplant.* **2004**, *13* (2), 169–177.

(12) Nagueh, S. F.; Shah, G.; Wu, Y. M.; Torre-Amione, G.; King, N. M. P.; Lahmers, S.; Witt, C. C.; Becker, K.; Labeit, S.; Granzier, H. L. *Circulation* **2004**, *110* (2), 155–162.
(13) Omens, J. H. *Prog. Biophys. Mol. Biol.* **1998**, *69* (2−3), 559–572.
(14) Mirsky, I.; Cohn, P. F.; Levine, J. A.; Gorlin, R.; Herman, M. V.; Kreulen, T. H.; Sonnenblick, E. H. *Circulation* **1974**, *50* (1), 128–136.
(15) Pego, A. P.; Grijpma, D. W.; Feijen, J. *Polymer* **2003**, *44* (21), 6495–6504.
(16) Foks, M. A.; Dijkhuis, K. A. J.; Grijpma, D. W.; Brouwer, L. A.; van Luyn, M. J. A.; Feijen, J. *J. Controlled Release* **2005**, *101* (1−3), 325–327.
(17) Pego, A. P.; van Luyn, M. J. A.; Brouwer, L. A.; van Wachem, P. B.; Poot, A. A.; Grijpma, D. W.; Feijen, J. *J. Biomed. Mater. Res.* **2003**, *67A* (3), 1044–1054.
(18) Pego, A. P.; Poot, A. A.; Grijpma, D. W.; Feijen, J. *Macromol. Biosci.* **2002**, *2* (9), 411–419.
(19) Zhang, Z.; Kuijer, R.; Bulstra, S. K.; Grijpma, D. W.; Feijen, J. *Biomaterials* **2006**, *27* (9), 1741–1748.
(20) Albertsson, A. C.; Eklund, M. *J. Appl. Polym. Sci.* **1995**, *57* (1), 87–103.
(21) Zhu, K. J.; Hendren, R. W.; Jensen, K.; Pitt, C. G. *Macromolecules* **1991**, *24* (8), 1736–1740.
(22) Tsutsumi, C.; Nakagawa, K.; Shirahama, H.; Yasuda, H. *Polym. Int.* **2003**, *52* (3), 439–447.
(23) Tsutsumi, C.; Nakagawa, K.; Shirahama, H.; Yasuda, H. *Macromol. Biosci.* **2002**, *2* (5), 223–232.
(24) Yoshii, F.; Darwis, D.; Mitomo, H.; Makuuchi, K. *Radiat. Phys. Chem.* **2000**, *57* (3−6), 417–420.
(25) Darwis, D.; Mitomo, H.; Enjoji, T.; Yoshi, F.; Makuuchi, K. *Polym. Degrad. Stab.* **1998**, *62* (2), 259–265.
(26) Abdel-Rehim, H. A.; Yoshii, F.; Kume, T. *Polym. Degrad. Stab.* **2004**, *85* (1), 689–695.
(27) Gan, Z. H.; Liang, Q. Z.; Zhang, J.; Jing, X. B. *Polym. Degrad. Stab.* **1997**, *56* (2), 209–213.
(28) Li, S. M.; Garreau, H.; Pauvert, B.; McGrath, J.; Toniolo, A.; Vert, M. *Biomacromolecules* **2002**, *3* (3), 525–530.
(29) Pitt, C. G.; Chasalow, F. I.; Hibionada, Y. M.; Klimas, D. M.; Schindler, A. *J. Appl. Polym. Sci.* **1981**, *26* (11), 3779–3787.
(30) Pitt, C. G.; Gratzl, M. M.; Kimmel, G. L.; Surles, J.; Schindler, A. *Biomaterials* **1981**, *2* (4), 215–220.
(31) *CRC Handbook of Chemistry and Physics*; Lide, D. R., Editor-in-Chief; CRC Press: Boca Raton, FL, 2002.
(32) Barltrop, J. A.; Owen, T. C.; Cory, A. H.; Cory, J. G. *Bioorg. Med. Chem. Lett.* **1991**, *1* (11), 611–614.
(33) Adler, G. *Science* **1963**, *141* (3578), 321–329.
(34) Brzozowski, A. M.; Derewenda, U.; Derewenda, Z. S.; Dodson, G. G.; Lawson, D. M.; Turkenburg, J. P.; Bjorkling, F.; Hugejensen, B.; Patkar, S. A.; Thim, L. *Nature* **1991**, *351* (6326), 491–494.
(35) Brady, L.; Brzozowski, A. M.; Derewenda, Z. S.; Dodson, E.; Dodson, G.; Tolley, S.; Turkenburg, J. P.; Christiansen, L.; Hugejensen, B.; Norskov, L.; Thim, L.; Menge, U. *Nature* **1990**, *343* (6260), 767–770.
(36) Marten, E.; Muller, R. J.; Deckwer, W. D. *Polym. Degrad. Stab.* **2003**, *80* (3), 485–501.

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