Synthesis, Characterization, and In Vitro Degradation of a Biodegradable Photo-Cross-Linked Film from Liquid Poly(ϵ-caprolactone-*co*-lactide-*co*-glycolide) Diacrylate

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There has been little study on the effect of composition or molecular weight on the biodegradation rate of photo-cross-linked biodegradable aliphatic polyesters though such information is important for tissue engineering scaffolds. We have synthesized a new series of photopolymerizable linear poly(ϵ-caprolactone-*co*-lactide-*co*-glycolide) diacrylates with different molecular weights (M_n = 1800, 4800, and 9300 Da) and compositions (20%, 40%, and 60% ϵ-CL) and studied their biodegradation rates. The resultant oligomers were amorphous and appeared as viscous liquids at room temperature. Liquid-to-solid polymerization was carried out by UV irradiation in the presence of a photoinitiator. The photocuring yield was high (greater than 95%), and the photo-cross-linked polymers were amorphous and rubbery. Mechanical measurements showed that the polymers can be stretchable or rigid; the high molecular weight/low ϵ-CL network has a strain of 176% and a modulus of 1.66 MPa while the low molecular weight/high ϵ-CL network has a strain of 21% and a modulus of 12.3 MPa. In a 10 week in vitro biodegradation study, the polymers exhibited a two-stage degradation behavior. In the first stage, the polymer weight and strain remained almost constant, but a linear decrease in the Young's modulus (E) and ultimate stress (σ) were observed. Lower oligomer molecular weight or ϵ-CL content correlated with a faster decrease in Young's modulus. In the second stage, which began when the Young's modulus dropped below 1 MPa, there was rapid weight loss and strain increase. The lower the ϵ-CL content, the earlier the second stage happened. Low molecular weight and high ϵ-CL content correlated with a longer modulus half-life (time for the modulus to degrade to 50% of its initial value). The degradation results suggest principles that may be helpful in predicting the biodegradation behavior of similar polymeric cross-linked networks. Films formed from these new polymers have excellent biocompatibility with smooth muscle cells.

1. Introduction

Linear thermoplastic polymers made from aliphatic polyesters, such as polyglycolide (PGA), polylactide (PLA), and poly(ϵ-caprolactone) (PCL), and their copolymers have been widely utilized as tissue engineering scaffolds, and their degradation behaviors have been well studied.[1−7] They degrade mainly by hydrolysis yielding hydroxyl carboxylic acids, which in most cases are nontoxic and ultimately metabolized by the body. PCL has high molecular (e.g., water, oxygen) permeability and is the most elastic polymer among the Food and Drug Administration (FDA)-approved aliphatic polyesters because of its low glass transition temperature (T_g) of −60 °C. (T_g's of PLLA and PGA are 55 and 38 °C, respectively.) Linear PCL is a rubbery semicrystalline (T_m = 55 °C) polymer at physiological temperature. However, PCL degrades very slowly and has thus been commonly copolymerized with other monomers for preparing a diversified family of copolymers that can be rubbery or glassy, slowly or quickly degrading, and rigid or highly flexible. Linear block copolymers with an intermediate to high amount of ϵ-CL (e.g., 55/45 mol % ϵ-CL/D,L-lactide[8]) are generally crystalline; amorphous ϵ-CL copolymer displays high mobility because of its low T_g and would not retain its shape at body temperature.[8,9] However, the crystalline copolymer degrades relatively slowly and inhomogeneously, which may not be favorable in some tissue engineering applications.

Photo-cross-linking is another route to produce amorphous copolymers of ϵ-CL capable of maintaining their shape at physiological temperature. Further, photo-cross-linkable liquid polymers can be micro- or nanopatterned for cell guidance.[10,11] The synthesis and photo-cross-linking of liquid biodegradable copolymers composed of ϵ-CL and trimethylene carbonate monomers end-capped with a coumarin[12] or phenylazide[13] functional group have been demonstrated. However, the cross-linking efficiency was generally low. Acrylate and methacrylate groups have been widely used for UV photopolymerization because of their high reactivity,[14] leading to rapid and near complete conversion. Meth(acrylated) poly(ϵ-caprolactone-*co*-D,L-lactide) which is star-shaped (rather than linear) has also been synthesized for tissue engineering or drug delivery applications.[8,11,15]

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Table 1. GPC, NMR, and DSC Analyses of PCLLGA Diacrylates

diacrylate	$M_{n,th}$[a]	[CL]/[LA]/[GA][b] monomer feed	[CL]/[LA]/[GA][b] polymer[c]	$M_{n,NMR}$[d] (Da)	M_n[e] (Da)	M_w[e] (Da)	D	viscosity (Pa s)
L-60	1800	60:20:20	62:18:20	1869	2080	3060	1.47	1.8 ± 0.1
M-60	4800	60:20:20	62:18:20	5529	7150	8810	1.23	21.6 ± 1.6
H-60	9300	60:20:20	59:21:20	10 193	12 200	15 700	1.28	95.5 ± 0.9
H-40	9300	40:30:30	45:27:28	9300	9850	12 400	1.26	361 ± 9
H-20	9300	20:40:40	23:38:39	9023	12 400	18 700	1.51	1506 ± 31

[a] Theoretical number average molecular weight. [b] Molar ratio. [c] Calculated from ^{1}H NMR. [d] Calculated from ^{1}H NMR using the H in the central tetra(ethylene glycol) block (“a” as designated in Figure 1) as the reference. [e] Measured using GPC.

Helminen et al.[8] and Amsden et al.[8,15] have reported on the degradation behaviors of photo-cross-linked meth(acrylated) aliphatic star polyesters. There is no report on the degradation behavior of photo-cross-linked diacrylated linear aliphatic polyesters. Degradation studies are typically tedious, but the ability to predict the degradation rate is highly useful. If the cross-linking density of photo-cross-linked biodegradable polyesters is not too high, then it is envisaged that the degradation rate will depend on the composition and molecular weight more like in the linear counterparts, opposite to the reported trend observed in cross-linked networks.

Scaffolds generally need to have not just sufficient initial but “partially degraded” elasticity.[16−18] The rate of degradation must also be tuned to match the regeneration of the new tissue. The scaffold should maintain its mechanical properties and structural integrity until the seeded cells proliferate sufficiently and produce enough extracellular matrix (ECM) and only then completely degrade and be absorbed by the body.[3,19] Control of the degradation rate and properties of the scaffold materials through tailoring the polymer composition is thus critical.

This study reports the design, synthesis, and characterization of a new series of liquid biodegradable random linear oligomers based on ϵ-CL, L-LA, and GA, which are end-capped with photo-cross-linkable acrylate groups. The diacrylated linear oligomers were designed with different molecular weights (1800, 4800, and 9300 Da) and compositions (by varying the ϵ-CL molar ratio, 20%, 40%, and 60%) so that the cross-linked polymers have varying mechanical properties and degradation rates. The high (9300 Da), medium (4800 Da), and low (1800 Da) molecular weight oligomers were denoted by H-*x*, M-*x*, and L-*x*, respectively, where *x* represents the % ϵ-CL content. Specifically, five oligomers (i.e., L-60, M-60, H-60, H-40, and H-20) were synthesized, and they have an equal molar ratio of GA to L-LA within each. The physical, chemical, and photo-cross-linking characteristics of the polymers were studied. The change of tensile properties, weight loss, and water absorption during degradation were systematically studied over a 10 week period. Biocompatibility of these polymers with rat smooth muscle cells (SMCs) was demonstrated by culturing on thin films of cross-linked polymers.

2. Materials and Methods

2.1. Materials. L-LA and GA were purchased from Purac Asian Pacific (Singapore) and recrystallized from ethyl acetate. ϵ-CL, acryloyl chloride, stannous octoate, and tetra(ethylene glycol) were obtained from Sigma-Aldrich and used as received. Triethylamine was purchased from Fluka and used as received. 2,2-Dimethoxy-2-phenylacetophenone (Irgacure 651) was obtained from CIBA chemicals. All other chemicals were of reagent grade and used without further purification. All cell culture reagents were obtained from Invitrogen unless otherwise specified.

2.2. Synthesis of Poly(ϵ-caprolactone-*co*-lactide-*co*-glycolide) Diacrylate. The poly(ϵ-caprolactone-*co*-lactide-*co*-glycolide) (PCLLGA) diols were prepared by ring-opening polymerization of ϵ-CL, L-LA, and GA, which was initiated with tetra(ethylene glycol) in the presence of stannous octoate as a catalyst. Predetermined amounts of tetra(ethylene glycol), L-LA, GA, ϵ-CL, and stannous octoate (controlled by the designed molecular weights and compositions as shown in Table 1) were added into a 100 mL round-bottomed flask. For example, for H-60, 4.32 g of L-LA, 3.48 g of GA, 20.52 g of ϵ-CL, 0.59 g of (3 mmol) tetra(ethylene glycol), and 0.03 g of stannous octoate were added, respectively. The reaction flask was equipped with a stirring bar and a high-vacuum stopcock that was connected to a dual bank manifold with one end connected to a vacuum pump and the other to argon gas. After repeated evacuation and purging with argon gas (three times), the reaction mixture was placed into an oil bath preheated to 145 °C, and polymerization was carried out under stirring for 24 h at 145 °C under argon atmosphere. The reaction mixture was cooled to room temperature, precipitated in hexane, and dried at 45 °C under reduced pressure to give a clear viscous liquid.

The isolated polymer was redissolved in dichloromethane in a three-neck round-bottomed flask and cooled to 0 °C in an ice bath. Acryloyl chloride and triethylamine dissolved in dichloromethane were added dropwise into the flask. For the H-60 example, 6 mmol of acryloyl chloride and 6 mmol of triethylamine were added. The mixture was reacted at 0 °C for 6 h and then at room temperature (∼25 °C) for 18 h. The mixture was washed with dilute HCl and $NaHCO_3$ solution to remove triethylamine and HCl, respectively. The mixture was then further washed with doubly distilled (dd) H_2O twice to remove traces of $NaHCO_3$. After this, the mixture was dried with anhydrous $MgSO_4$ and microfiltered. Dichloromethane was then removed by rotary evaporation, and the residual amount was removed under reduced pressure at 65 °C for 48 h. The obtained diacrylated oligomers were colorless or clear light yellow viscous liquids. The triethylamine should be added slowly dropwise into the reaction flask; otherwise the reaction mixture will become yellowish or even brownish, which will cause poor UV transmission and autofluorescence. The latter will cause difficulties in cell characterization where fluorescence microscopy is frequently used.

2.3. Chemical and Thermal Characterizations. ^{1}H NMR spectra were recorded on a Bruker DMX-300 spectrometer, working at 300.130 MHz. The solvents for ^{1}H NMR measurement were $CDCl_3$ and acetone-d_6. The solvent contained 0.5% tetramethylsilane as the internal standard.

Number and weight average molecular weights (M_n and M_w, respectively) and molecular weight distribution (M_w/M_n) were determined by gel permeation chromatography measured with an Agilent 1100 series equipped with gel permeation chromatography−size exclusion chromatography (GPC-SEC) data analysis software. Samples were analyzed at 25 °C with tetrahydrofuran as the eluent at a flow rate of 1.0 mL min^{-1}. Polystyrene standards were used.

Fourier transform infrared (FTIR) spectra of the oligomers were recorded on a Nicolet Magna-IR 560 spectrometer equipped with a Golden Gate single reflection diamond attenuated total reflection (ATR) unit over the wavelength range of 4000−400 cm^{-1}. The spectra were

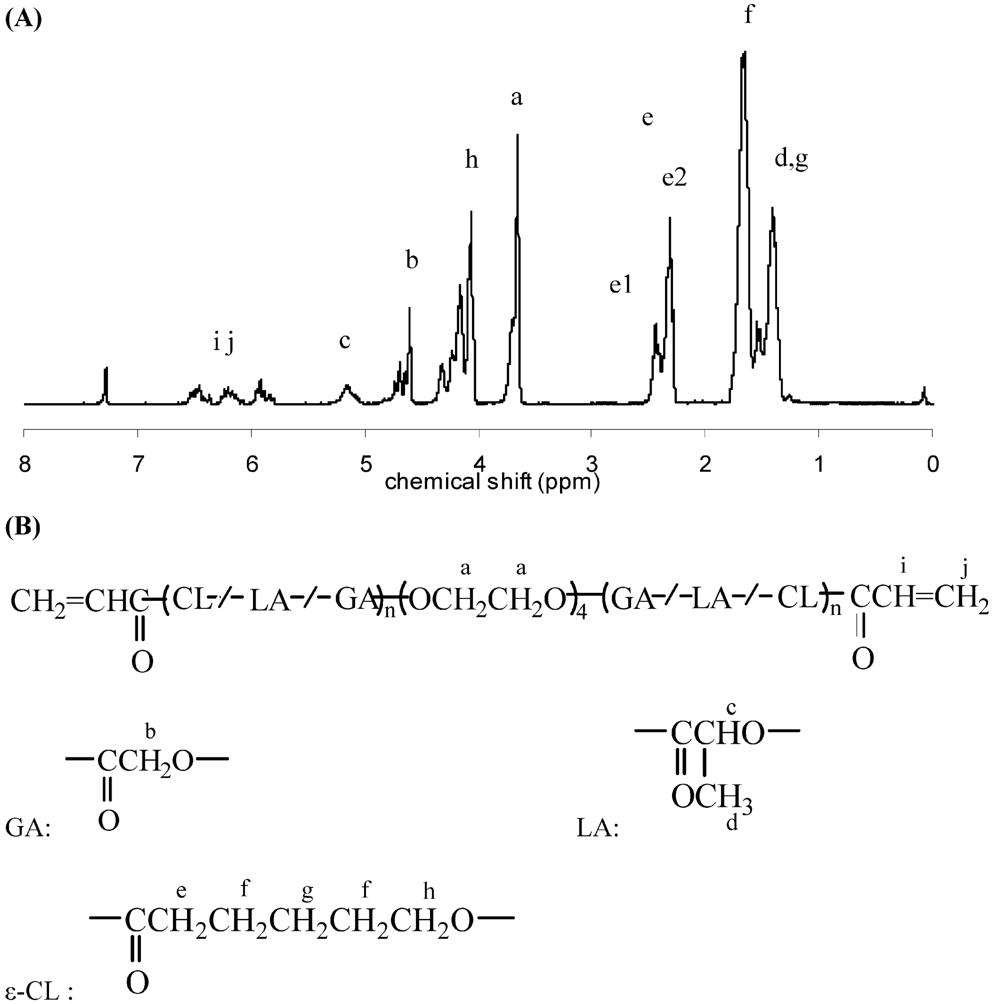


Figure 1. (A) ^{1}H NMR spectrum of PCLLGA diacrylate oligomer (L-60). (B) Molecular structure of PCLLGA diacrylate.

collected with a resolution of 4 cm^{-1} and a scan number of 32 using a mercury cadmium telluride (MCT) detector.

Differential scanning calorimetry (DSC) measurements were carried out using a TA DSC 2920 modulated differential scanning calorimeter. The cross-linked sample (section 2.5) was heated from room temperature (at 20 °C/min) to 200 °C, which was maintained for 1 min. It was cooled at 10 °C/min to −60 °C, which was maintained for 1 min. It was then reheated at 20 °C/min to 200 °C. The oligomers were heated to 80 °C only, and for the three oligomers made with 60% ϵ-CL, due to their low T_g's reaching the lower temperature limit of the instrument (−60 °C), after the first heating scan, the samples were cooled to −100 °C by manually adding liquid nitrogen and reheated at 20 °C/min to 80 °C. All DSC data presented here are from the second heating scans.

Viscosity of the diacrylates was measured using a controlled strain rheometer (ARES Rheometric Scientific) using a 25 mm diameter cone. The rheometer was calibrated using Fluka silicone oil (DC200), which has a reported viscosity of 100 mPa s at 25 °C under the shear rate of 10 rad/s. The viscosities of the diacrylates reported were those measured at 10 rad/s and 25 °C.

2.4. Curing Analyzed by FTIR Spectra. Each of the diacrylated oligomers was stirred for 24 h at 65 °C with 0.5 wt % of Irgacure 651 photoinitiator that was predissolved in butanone (10:90 (w/w) Irgacure 651/butanone); the excess butanone was removed under reduced pressure at 65 °C for 2 h. The dynamic FTIR spectra of photopolymerizing resins were recorded with a Nicolet Magna-IR 560 spectrometer equipped with an MCT detector. A single reflection diamond ATR device was used for sampling. UV irradiation was performed with a mercury lamp. The light was guided to the sample using a fiber-optic cable with an inner diameter of 1 cm. The 365 nm intensity of the UV light at the sample on the diamond crystal was adjusted to approximately 10 mW/cm^2.[20] The spectra were continuously collected during the dynamic UV curing process. The kinetic conversion was calculated from the decay of the absorption bands of the $CH{=}CH_2$ deformation mode at 810 cm^{-1} by integration of the peak areas. The symmetrical stretch vibration of $-CH_3$ at 2862 cm^{-1} was used as a reference signal to compensate for differences in sample thickness since this peak remained constant throughout the course of the experiments. The conversion was calculated from the following equation

$$\text{conversion (\%)} = 1 - (A_t/B_t)/(A_0/B_0) \qquad (1)$$

where A_t and B_t are the areas under peaks at 810 and 2862 cm^{-1} at time t, respectively, and A_0 and B_0 are the areas under peaks at 810 and 2862 cm^{-1} at time 0.

2.5. Mechanical Testing of Cross-Linked Films. To make the cross-linked films, the UV resin formulation was sandwiched between two pieces of glass with a 125-μm-thick spacer and photopolymerized using a flood UV exposure system equipped with a 350 W mercury lamp with a 365 nm intensity of 10 mW/cm^2. The cross-linked polymer samples were cut into dumbbell shapes (according to ASTMD-1457), which were 14 mm long and 5 and 16 mm wide at the narrow and gripping sections, respectively. An Instron 5569 equipped with Merlin Software and a static 10 N load cell was used. The samples were pulled at 2 mm/min until breakage occurred. Only those samples breaking at the narrow region were included in the analysis, and the test results reported are averages of 5−10 valid measurements.

2.6. In Vitro Degradation Study of Photocured Films. The initial weights (W_0) of the photocured films were recorded. The photocured films were then incubated in phosphate-buffered saline (PBS, pH 7.4)

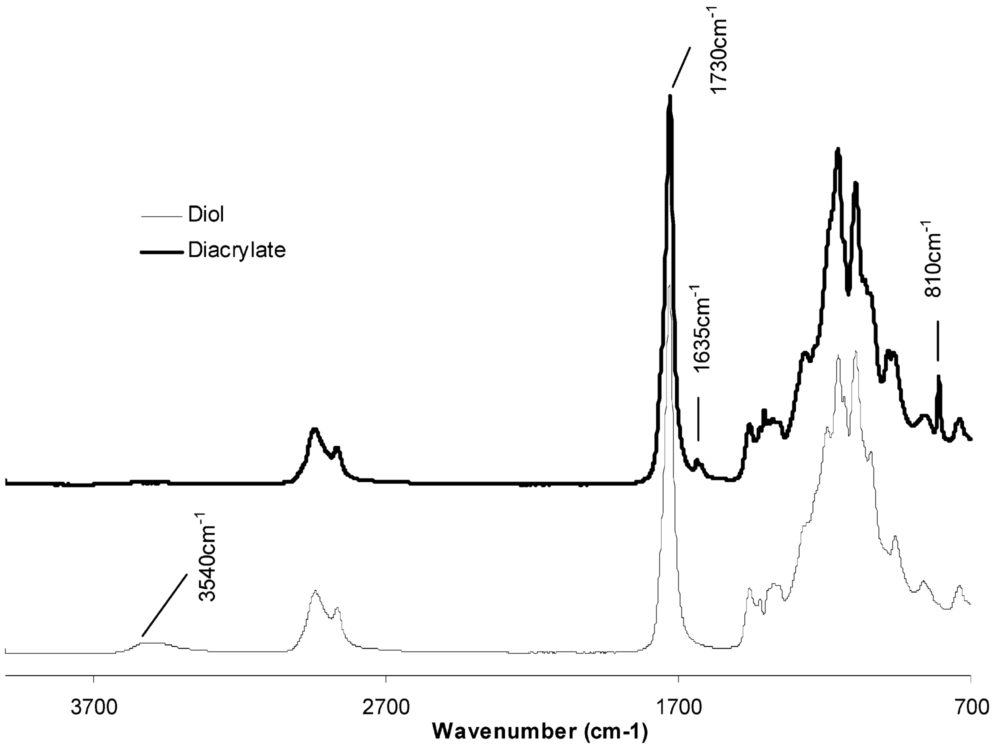


Figure 2. FTIR spectrum of PCLLGA diol and diacrylate (L-60).

at 37 °C for up to 10 weeks in an orbital shaking machine with a shaking speed of 50 rpm. The pH of the solution was checked weekly, and the solution was replaced when the pH dropped below 7.0. After 1, 2, 4, 6, 8, and 10 weeks, the swollen weight (W_S) and dried weight (W_D) were measured after wiping the surface water with paper and after vacuum-drying, respectively. The weight loss (WL) and water absorptivity (WA) were calculated using eqs 2 and 3, respectively. The results reported are averages of three measurements

$$\mathrm{WL}\ (\%) = 100(W_0 - W_D)/W_0 \qquad (2)$$

$$\mathrm{WA}\ (\%) = 100(W_S - W_D)/W_D \qquad (3)$$

The tensile properties of the control and degraded films at these intervals were also measured as described in section 2.6.

2.8. Cell Culture on Photocured Films. SMCs used were from rat aorta (ATCC, CRL-1444). SMCs were maintained in 75 cm^2 tissue culture polystyrene flasks in complete growth medium (Dulbecco's modified Eagle's medium with 4 mM L-glutamine supplemented with 10% fetal bovine serum, 50 IU/mL of penicillin, and 50 μL/mL of streptomycin). Cells were passaged by trypsinization with 0.25% (w/v) trypsin−0.03% (w/v) EDTA solution before reaching confluence, usually every third day. The different polymer films were cut into 1.5 cm diameter discs and sterilized in 70% ethanol/30% dd H_2O solution for 24 h. Before cell seeding, the films were moved to a 24-well culture plate and equilibrated with PBS for 30 min. Then SMCs together with culture medium were added to the plate (10 000 cells/cm^2) and incubated for 3 days. Cell density after 1 day and 3 days of culturing was assessed by cell counting using a hemocytometer after detachment from the substrates and trypan blue staining. Results reported are averages from three measurements. Phase contrast images were taken using a Zeiss inverted microscope.

3. Results and Discussion

3.1. Synthesis and Characterization of Un-Cross-Linked Oligomers. The five diacrylated oligomers synthesized were characterized by ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm). As an illustration, the spectrum of the diacrylated oligomer L-60 is shown in Figure 1a. The total proton assignment of L-60 in the ^{1}H NMR spectrum is specified in Figure 1b: **(I)** $CH_2{=}CH$ vinyl groups at 5.84−6.54 ppm (peaks i and j), **(II)** CH_2CH_2 groups attached to oxygen atoms at 3.65 ppm (peak a), **(III)** CH tertiary carbon groups attached to oxygen atoms and carbonyl groups at 5.11 ppm due to the LA moiety as a broad peak (c), **(IV)** lateral methyl CH_3 groups and the middle CH_2 groups in $CH_2CH_2CH_2$ groups at 1.38−1.65 ppm (peaks d and g), **(V)** CH_2 groups attached to oxygen atoms at 4.04−4.32 ppm (peak h), **(VI)** CH_2 secondary carbon groups attached to oxygen atoms and carbonyl groups at 4.60−4.73 ppm (peak b) due to the GA moiety, **(VII)** CH_2 groups attached to carbonyl groups at 2.31−2.46 ppm (peak e), and **(VIII)** two side CH_2 groups in $CH_2CH_2CH_2$ groups at 1.35−1.68 ppm (peak f). Some peaks can be further split due to the different stereosequences.[21] For example, peak e was split into e1 and e2 doublets corresponding to $COCH_2$ groups attached to the GA or LA units (e1), which are more polar than ϵ-CL unit, and those attached to another ϵ-CL repeat unit (e2), respectively. However, clear elucidation of the stereosequences of the oligomers requires high-resolution scanning. Pack et al.[21] used 600 MHz ^{1}H NMR scanning. The molar ratios of ϵ-CL, LA, and GA in each synthesized diacrylate were calculated according to the relative intensities of the corresponding protons and were similar to the designed values (Table 1).

Figure 2 shows the typical FTIR spectra of the PCLLGA diol and diacrylate using L-60. The peak at 3540 cm^{-1} due to the hydroxyl group in the diol disappeared completely after the acrylation reaction, suggesting complete or nearly complete conversion of the diol to the diacrylate. The $CH{=}CH_2$ group gave two absorption bands at 810 cm^{-1} (C=C bond twisting) and 1635 cm^{-1} (C=C bond stretching), confirming that the acrylation was successful. The absorption bands at 1730 cm^{-1} are attributed to ester stretching in the oligomer backbone.

GPC results (Table 1) gave a narrow and normal distribution (1.23−1.51) of the molecular weight. The measured number

Table 2. DSC, Real-Time FTIR, and Mechanical Measurement Results

	L-60	M-60	H-60	H-40	H-20
T_g/diacrylate (°C)	−57.1	−54.2	−42.3	−36.6	−12.8
T_g/cross-linked (°C)	−42.2	−47.2	−39.5	−26.9	−0.4
ΔT_g (°C)[a]	14.9	7.0	2.8	9.7	12.4
final conversion (%)[b]	99	99	97	98	98
$t_{1/2}$ (s)	4.2	8.6	11.6	15.7	17.5
E (MPa)	12.29 ± 0.47	6.40 ± 0.49	3.36 ± 0.10	2.73 ± 0.05	1.66 ± 0.11
σ (MPa)	2.46 ± 0.26	3.76 ± 0.47	2.83 ± 0.11	2.69 ± 0.20	2.39 ± 0.21
ϵ (%)	21 ± 2	79 ± 12	129 ± 3	137 ± 12	176 ± 11

[a] $\Delta T_g = T_g$/cross-linked (°C) − T_g/diacrylate (°C). [b] The final conversion was the conversion calculated after 5 min of UV irradiation.

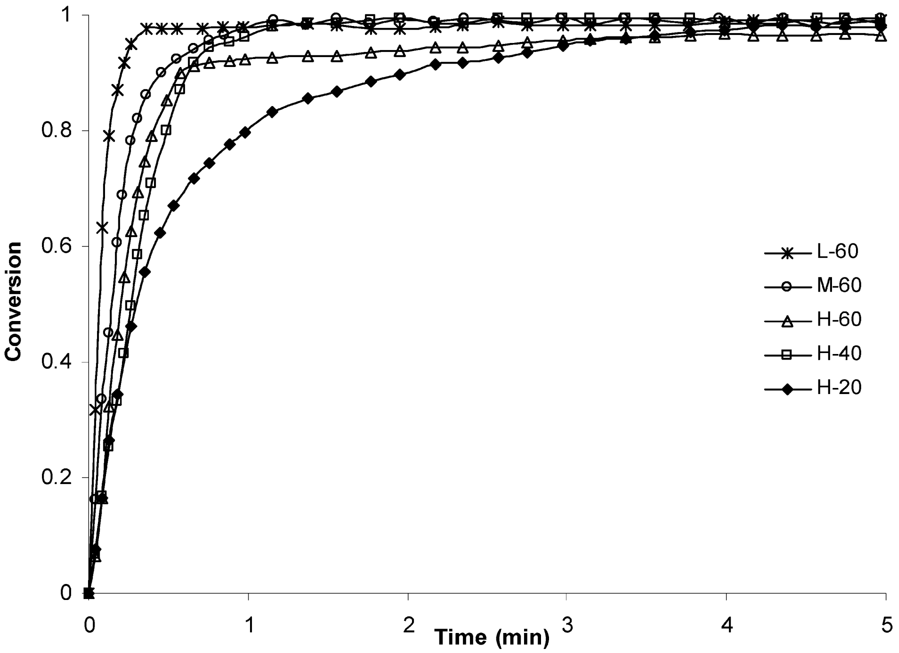


Figure 3. Photocuring yield of PCLLGA diacrylates.

average molecular weights (M_n) were similar to the designed M_n, indicating that the polymerization proceeded to near completion.

The viscosity of the diacrylate was observed to decrease as the ϵ-CL content increases but increases with increased molecular weight (Table 1). With the molecular weight kept almost constant, the viscosity decreases significantly from 1506 Pa s with H-20 (low ϵ-CL content) to 96 Pa s with H-60 (high ϵ-CL content). The viscosity increases from 1.8 Pa s with L-60 to 95.5 Pa s for H-60 as the molecular weight increased from 1800 to 9300 Da (ϵ-CL kept at 60 mol%).

DSC scans of the (un-cross-linked) diacrylates show no melting endotherms, indicating the absence of crystalline domains (data not shown). T_g of the amorphous oligomers decreases from −12.8 (H-20) to −42.3 °C (H-60) with an increasing ϵ-CL molar ratio (with the molecular weight kept almost constant) (Table 2). But T_g increases from −57.1 to −42.3 °C as the molecular weight increases from 1800 to 9300 Da (ϵ-CL kept at 60 mol%, Table 2). Unlike other high molecular weight (M_n > 150 000 Da) ϵ-CL/LA copolymers,[22] our oligomers displayed one clear single glass transition peak, which may be due to the relatively low molecular weight preventing blocky structure formation.

3.2. Photocuring Characteristics. Figure 3 shows the dynamic acrylate conversion versus UV exposure time for different PCLLGA diacrylates. The final conversion and time to reach 50% of final conversion ($t_{1/2}$) are summarized in Table 2. The reactions were nearly completed after 1 min of UV irradiation for all oligomers except H-20, which had the highest viscosity (Table 1). Free radical polymerization kinetics are known to be controlled by diffusion.[14] The high viscosity of H-20 can accelerate the diffusion-controlled propagation, causing enhanced auto-decceleration so that the reaction is slowed down.[14,23,24] However, all formulations were highly converted with prolonged UV irradiation, with final conversions reaching more than 95% after 5 min of UV irradiation, so leaching of unreacted oligomers prior to cell culturing was not necessary. With deceasing molecular weight, the reaction proceeded faster due to the higher concentration of functional groups: $t_{1/2}$ is 4.2 s for L-60 and increased to 11.6 s for H-60 (Table 2). The 10 min interval used in the preparation of photocured films for testing was confirmed to be sufficient.

3.3. Thermal and Tensile Properties of Photo-Cross-Linked Films. The thermal behavior of the photo-cross-linked polymers was also examined with DSC, and the results are summarized in Table 2. Cross-linking led to an increase of the glass transition temperature (ΔT_g) compared to those of the un-cross-linked oligomers. ΔT_g ranged from 2.8 to 14.9 °C for H-60 and L-60, respectively. This trend is accounted for by the higher cross-linking density and tighter network formed from oligomers with lower molecular weights. In addition, the size of the T_g increase was found to anticorrelate with the ϵ-CL content among the high molecular weight oligomers/polymers. When more GA and LA monomers were present (keeping the same molecular weight), the chain−chain interaction is increased due to increased chain polarity, so the cross-linking that brings chains

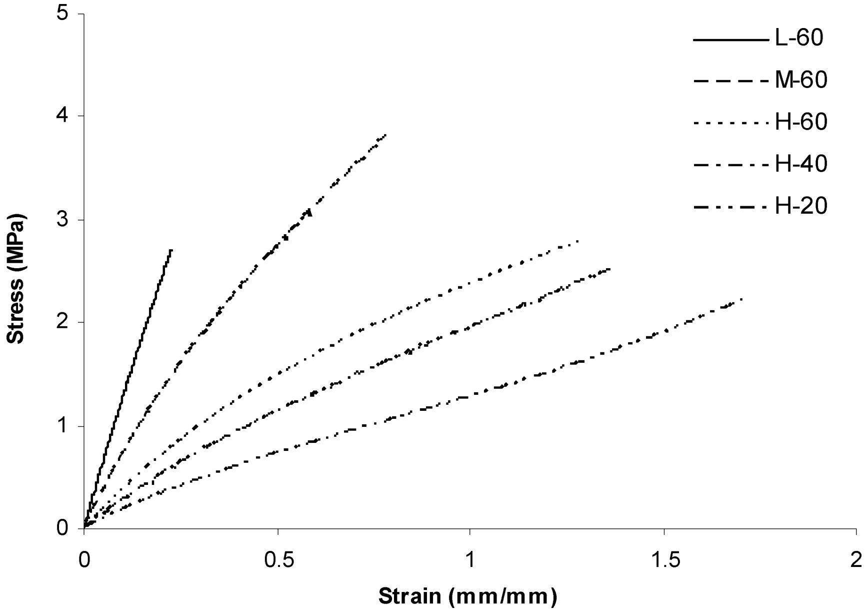


Figure 4. Representative stress−strain curves of cross-linked PCLLGA polymers.

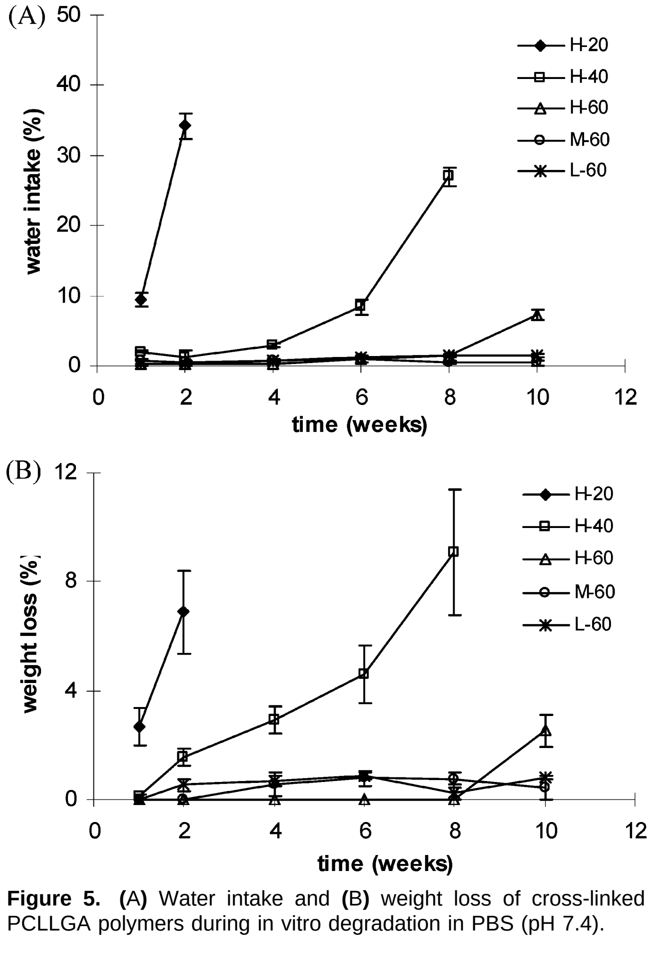


Figure 5. (A) Water intake and (B) weight loss of cross-linked PCLLGA polymers during in vitro degradation in PBS (pH 7.4).

to closer proximity has a more pronounced effect on the ΔT_g of more polar oligomers. All of the cross-linked polymers had glass transition temperatures well below body temperature.

Representative uniaxial tensile stress−strain curves of the cross-linked PCLLGA copolymers are shown in Figure 4. Average values for the tensile Young's modulus (E), ultimate stress (σ), and maximum strain (ϵ) are listed in Table 2. A rather similar ultimate tensile strength was measured for all formulations (2.39−3.76 MPa). However, there is a substantial range of Young's modulus (6.4 fold increase from the lowest to the highest) and the maximum strain in these samples (7.4 fold increase from the lowest to the highest). The cross-linked PCLLGA polymers were generally elastomeric with a high maximum strain exceeding 50% except for L-60. The very stiff L-60 has a low strain at failure, and it is less suitable for applications in which significant flexibility is desired, such as arterial grafts. As the molecular weight of the oligomer decreases from 9300 Da for H-60 to 1800 Da for L-60, the corresponding cross-linking density increases so that the polymer becomes stiffer and less extensible. However, the ultimate stress does not change much with variation of the molecular weight. The effect of ϵ-CL content on the tensile properties was not significant.

3.4. In Vitro Degradation Study. Theoretically, we expect both the ϵ-CL content and network density to affect the water uptake and degradation rate. As the relatively hydrophobic ϵ-CL content increases, the CL/GA/LA copolymer is expected to degrade more slowly. Also, as the cross-link density of the polymer increases, diffusion of water into the cross-linked rubbery networks decreases so that the degradation rate should anticorrelate with the cross-link density of the network. Our experimental results confirm these two effects.

Figure 5 shows that cross-linked samples made from H-20 that had the lowest ϵ-CL content and cross-link density of the five samples had the quickest biodegradation and water intake rates. After 2 weeks of immersion in PBS, the H-20 cross-linked samples had absorbed much water (34%) and had lost approximately 7% of their original weight (Figure 5). After 4 weeks of immersion in PBS, they had become pastelike with no definable form, so accurate weighing of the samples became impractical; the degradation study for this polymer was then stopped. Samples made from cross-linked H-40 with intermediate molecular weight had the second highest biodegradation and water absorption rates; H-40 samples absorbed 27% water and lost 9% weight after 8 weeks of immersion in PBS; by week 8, they become too weak to withstand the pressure of the clamps in the tensile testing machine, so mechanical testing was stopped, and after 10 weeks of immersion in PBS, the H-40 samples disintegrated. The H-60 polymers were the most durable of the H-series polymers, absorbing less water (7%) and losing less

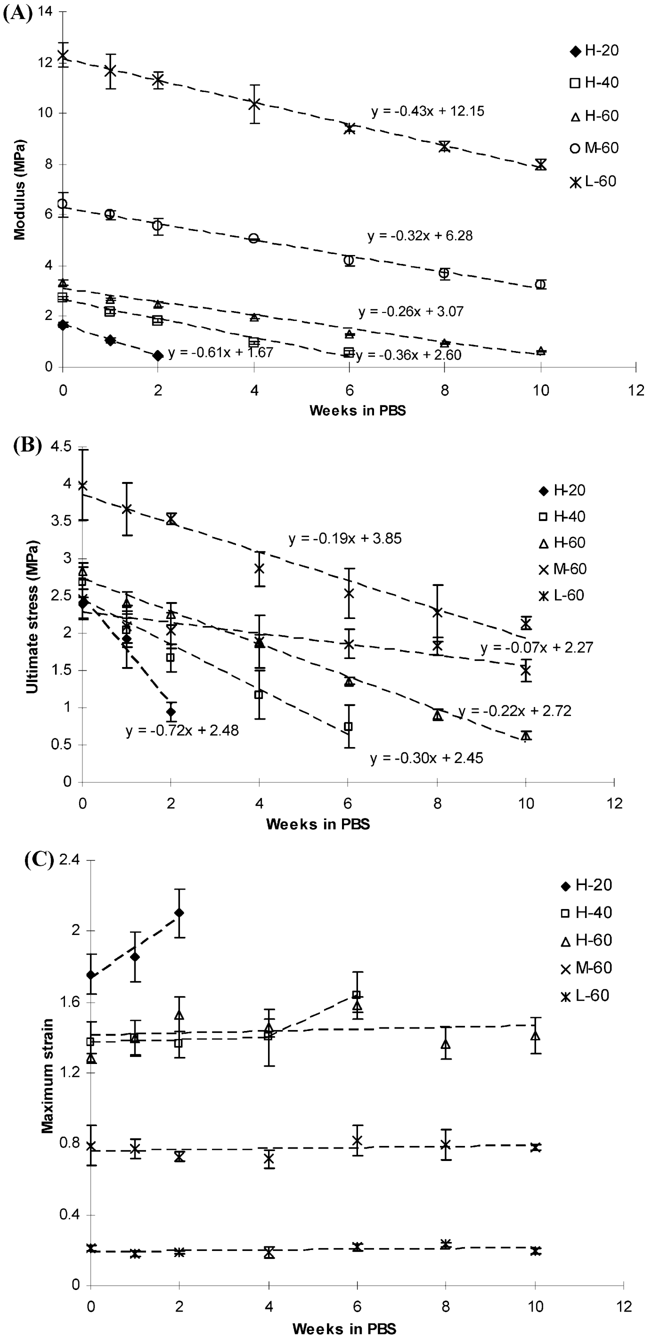


Figure 6. Change in mechanical properties of cross-linked PCLLGA polymers during in vitro degradation in PBS (pH 7.4): **(A)** Young's modulus, *E*, **(B)** ultimate stress, σ, and **(C)** maximum strain, ϵ.

weight (2.5% of the initial weight) after a longer (10 weeks) immersion in PBS.

Figure 5 also shows that for M-60 and L-60 there were little water intake and weight loss observed during the 10 week period as these polymers have both higher cross-linking density and higher ϵ-CL content.

Figure 6 shows the changes in the mechanical properties (*E* and σ) of the cross-linked polymers with respect to time during in vitro degradation. A linear decrease in the Young's modulus (*E*) and ultimate stress (σ) was observed regardless of the polymer composition, indicating a bulk erosion mechanism, resembling the behavior of high molecular weight linear biodegradable polyesters. With a surface-eroding polymer, erosion only occurs at the surface of the sample so that generally the mechanical properties will be maintained.[25,26]

Table 3. Linear Regression Coefficients for Young's Modulus (*E*) and Ultimate Stress (σ) and A_E, A_σ, E_0, and σ_0, and "Half-Decay Lifetime" ($A_E/2E_0$) Values for PCLLGA Polymers During Degradation in Vitro in PBS (pH 7.4)[a]

	L-60	M-60	H-60	H-40	H-20
A_E (MPa/week)	0.43	0.32	0.26	0.36	0.61
E_0 (MPa)	12.15	6.28	3.07	2.60	1.67
A_σ (MPa/week)	0.07	0.19	0.22	0.30	0.70
σ_0 (MPa)	2.27	3.85	2.72	2.45	2.48
$\tau_{1/2}$ (week)	14.1	9.8	5.9	3.6	1.4

[a] $X(t) = -A_x t + X_0$ (X = A, σ); t is the immersion time in PBS in weeks.

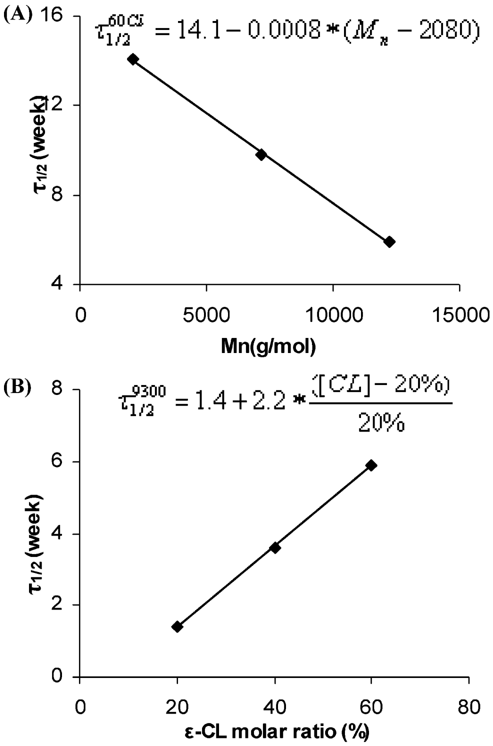


Figure 7. Modulus half-life of PCLLGA diacrylates **(**A**)** with fixed ϵ-CL content (60%) versus measured M_n, and **(**B**)** with roughly fixed molecular weight (9300 Da) versus ϵ-CL content.

We represent the degradation of Young's modulus or ultimate stress with time by

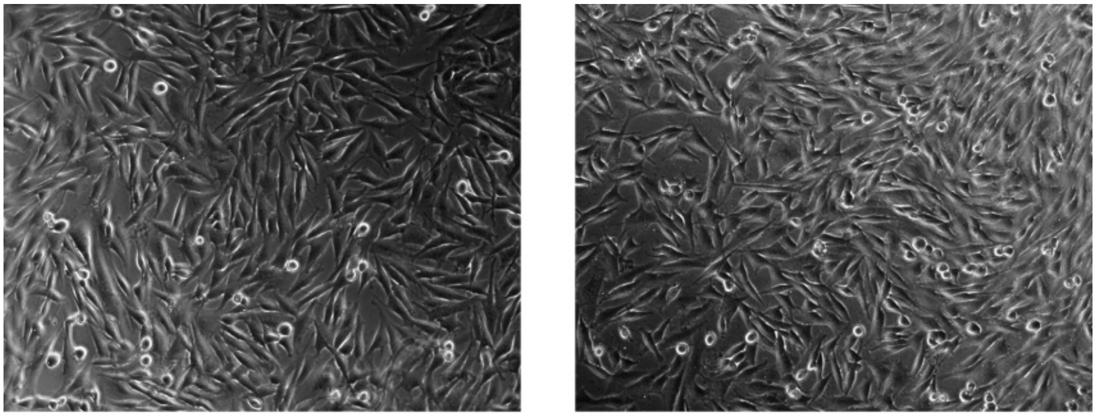
$$E(t) = -A_E t + E_0 \tag{4}$$

and

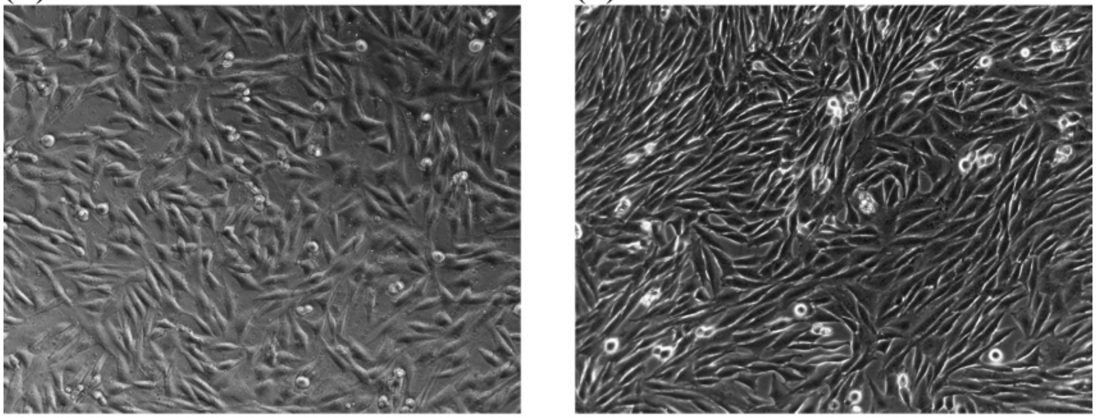
$$\sigma(t) = -A_\sigma t + \sigma_0 \tag{5}$$

where *t* (in weeks) is the immersion time in PBS.

The values of A_E, A_σ, E_0, and σ_0 for each cross-linked polymer based on linear regression fit to the experimental data are summarized in Table 3 and also noted in Figure 6. The values of E_0 and σ_0 obtained from extrapolation to zero time (Table 3) are generally similar to *E* and σ summarized in Table 2. As the amount of ϵ-CL increased, the rate of decrease in Young's modulus (A_E) and ultimate stress (A_σ) were found to decrease: A_E is 0.61 MPa/week for H-20 and decreases to 0.26 MPa/week for H-60. This correlation of the degradation rate of tensile properties with ϵ-CL content is similar to the trend noted for the water absorption/weight loss (Figure 5). The Young's modulus was also found to degrade faster as the molecular weight between cross-links decreases: A_E is 0.26 MPa/week



(A) L-60 **(B)** H-60



(C) H-20 **(D)** Tissue culture plate

Figure 8. Phase contrast images of SMCs grown on different films and a TCPS after 3 days of culturing.

for H-60 versus 0.43 MPa/week for L-60. The ultimate stress degraded faster with increased molecular weight: A_σ is 0.22 MPa/week for H-60 versus 0.07 MPa/week for L-60.

Figure 6C shows the change in maximum strain with increased PBS immersion time. Strain is less sensitive to degradation for these cross-linked polymers. No significant changes in maximum strains were found except for H-20 and H-40. For H-20, the strain was increased from 176% to 210% (an increase of 19%) over 2 weeks; after the 2 week period, the samples absorbed significant water and disintegrated (as shown in Figure 5A). Additionally for H-40, the maximum strain increased by 17% from week 4 to week 6 though little change was noted before the 4 week period. The maximum strain of an elastomer depends on the distribution of end-to-end distances between cross-link points within the matrix[27] so that the distance between the cross-link points did not change much when only some chains had been cleaved. However, as more chains were cleaved, the cross-linked polymer became somewhat linear so that the maximum strain increased. A relatively stable strain for lightly degrading cross-linked biodegradable polyesters has also been observed with star-shaped acrylated aliphatic polyesters.[8,15] The retained extendibility of partially degraded cross-linked copolymers was a significant improvement over the corresponding linear copolymers. With the latter, it has generally been observed that extendibility decreases when molecular weight decreases, and this happens usually from the initial period of immersion.[5,6,28] The retained initial extendibility during the first stage of degradation (from weeks to months) of our cross-linked polyesters is useful in engineering soft tissues in applications in which the scaffold must be flexible during tissue regeneration.

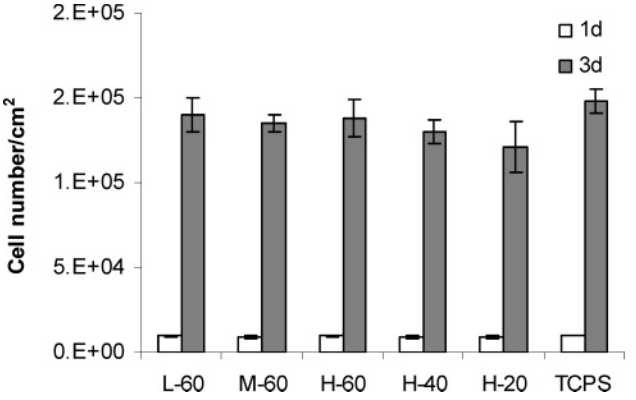


Figure 9. SMC density on different films and a TCPS at day 1 and day 3.

The degradation rate or extent of our cross-linked system can be tuned by varying the molecular weight or composition. It appears that the acidic degradation products can be easily diffused from these networks so that lower network density or ϵ-CL content causes faster degradation; thus the degradation of our linear cross-linked polyesters can be easily tuned via manipulation of molecular weight or composition.

From Figures 6A and 6B, the Young's modulus and ultimate stress of H-20, H-40, and H-60 dropped to below 1 MPa after 2, 6, and 10 weeks respectively; by then, these polymers also absorbed water quickly, lost weight rapidly (Figure 5), and became pastelike. This suggests that the degradation of these networks can be roughly divided into two stages. The first stage is characterized by mechanical weakening demonstrated by a linear decrease in Young's modulus and ultimate stress with almost constant maximum strain and insignificant water absorption/weight loss. The second stage beginning after the Young's modulus and ultimate stress have dropped to below 1 MPa is accompanied by rapid weight loss of the polymer, which may be attributed to its loose network with rapid water penetration. By extrapolation, E and σ are estimated to drop below 1 MPa simultaneously after 17 and 26 weeks for M-60 and L-60, respectively. Also, for each of the networks, the ultimate stress and Young's modulus degrade to approximately 1 MPa almost simultaneously.

To further estimate the degradation process, the "modulus half-life" defined as the "time required for Young's modulus to decline to half of its initial value" was also computed for the five copolymers (Table 3) according to

$$\tau_{1/2} = \frac{E_0 - E_0/2}{A_E} = \frac{E_0}{2A_\mathrm{E}}\ \text{(weeks)} \qquad (6)$$

Figure 7A shows the correlation between $\tau_{1/2}$ and ϵ-CL content for the three high molecular weight oligomers (H-60, H-40, and H-20). The "modulus half-life" obeys a linear relationship versus their ϵ-CL content (admittedly there are only three points) (Figure 7A) that can be represented by

$$\tau_{1/2}^{9300} = 1.4 + 2.2\,\frac{([\mathrm{CL}] - 20\%)}{20\%}\ \text{(weeks)} \qquad (7)$$

where [CL] is the molar ratio of ϵ-CL. Using eq 7, we can predict that H-80 would have a modulus half decay time of approximately 8 weeks. Equation 7 is not quite a kosher relationship, though, since the actual M_n is not constant (Table 1). Similarly, for the series of polymers with fixed ϵ-CL content, the "modulus half-life" also obeys a linear relationship versus their measured M_n values (Figure 7B) as follows

$$\tau_{1/2}^{60\mathrm{CL}} = 14.1 - 0.0008(M_\mathrm{n} - 2080)\ \text{(weeks)} \qquad (8)$$

From the above, we can predict how long the scaffolds made from these copolymers can retain their mechanical/structure integrity, and this is important for tissue engineering scaffolds.

3.5. In Vitro Biocompatibility with Smooth Muscle Cells. The cross-linked films were sterilized by soaking in 70% ethanol/30% dd H_2O solution. The in vitro biocompatibility of the sterilized films was tested using a SMC line. After 3 days of culturing, SMCs were found to grow to near confluence on these polymer films, with cell organization and morphology resembling that on a tissue culture plate (Figure 8). The cell density on these polymer films was also found to be comparable to that on a tissue culture plate (89−99% of that on a tissue culture polystyrene plate (TCPS) at day 1 and 82−95% of that on a TCPS at day 3 (Figure 9)). These cross-linked polymers are biocompatible and are expected to be useful as tissue engineering scaffold materials.

4. Conclusion

A new series of biodegradable photopolymerizable linear PCLLGA diacrylates with different molecular weights and different compositions were successfully synthesized. The obtained oligomers and its corresponding cross-linked networks were found to be amorphous with low T_g's.

The oligomers reacted via photopolymerization to form highly cross-linked networks within minutes upon UV irradiation, and the obtained polymer films have higher than 95% conversion and were transparent and rubbery. The networks mostly have high elongations at breakage (more than 50%), and the modulus vary from 12.3 to 1.7 MPa.

The degradation results suggest a two-stage degradation behavior. In the first stage, Young's modulus and the ultimate stress were found to decrease linearly with time for all five networks. Lower molecular weight and lower ϵ-CL content led to a faster decrease in Young's modulus. However, the cross-linked polymers maintained their initial extendibility (maximum strain) during this stage. This is an improvement over linear copolymers whose extendibility has been found to decline during degradation and is a desirable feature since the partially degraded scaffold must often be flexible during tissue regeneration. The second stage of degradation began after Young's modulus and the ultimate stress had dropped to approximately 1 MPa and was characterized by rapid weight loss and water uptake. The lower the ϵ-CL content and the higher the molecular weight, the earlier the second stage occurred. By variation of the molecular weight and the composition, the cross-linked polymers can be adjusted to maintain their tensile properties for time periods ranging from several weeks to several months. This flexibility should make these networks attractive materials for a wide range of biomedical applications. The linear relationship between composition or M_n and the modulus half-life suggests that the lifetime should be easily predictable.

SMC culturing showed that these cross-linked polymers are highly biocompatible, nearly as good as tissue culture polystyrene, and they can be expected to be useful as tissue engineering scaffolds.

Through adjustment of the composition and molecular weight of a diacrylated aliphatic polyester, cross-linked networks with different mechanical properties but controllable biodegradation rates can be designed to meet various requirements in tissue engineering, especially soft tissue engineering applications. The results may also be helpful in predicting the degradation behavior of similar polymer networks.

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