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Anti-atherosclerotic peptide delivery from a photocrosslinkable biodegradable network

Brian Amsden*, Bo Qi

Department of Chemical Engineering and Human Mobility Research Centre, Queen's University, 19 Division St., Kingston, Ontario, Canada K7L 3N6

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ABSTRACT

A water-soluble, 19-mer peptide fragment of serum amyloid A called MFFD is being examined as a possible treatment for atherosclerosis. As a means of administering this drug in a sustained fashion through subcutaneous implantation, a biodegradable network formulation was prepared. The formulation consisted of 1000 and 4000 Da α,ω-diacrylate *oligo*(D,L-lactide)-*b*-poly(ethylene glycol)-*b*-oligo(D,L-lactide) (DLPEGDLDA) copolymerized with 2700 and 5000 Da ω,ω,ω-triacrylate star-poly(ε-caprolactone-*co*-D,L-lactide) using UV irradiation. The influence on the network properties and degradation rate of the network on the amount and type of DLPEGDLDA copolymerized with the two different molecular weight ASCPs were examined *in vitro*. The networks degraded by bulk hydrolysis at a rate controlled primarily by the molecular weight of the ASCP used. Nevertheless, all the networks were completely degraded within 16 weeks. The MFFD was released in a diffusional manner at a rate influenced by the degree of swelling of the network and the molecular weight of the ASCP used; using an ASCP of a lower molecular weight for a given DLPEGDLDA resulted in a slower release rate. The degree of swelling of the networks was controlled solely by the nature of the PEG used in preparing the DLPEGDLDA, with greater swelling observed with higher PEG molecular weight and for greater amounts of PEG incorporation. The MFFD was not degraded during the photocrosslinking reaction or by potential acidic degradation products that may have accumulated within the device. This formulation provides a means of achieving a desirable release rate from a degradable, water-swellable network through selection of ASCP molecular weight and DLPEGDLDA composition.

1. Introduction

Atherosclerosis is a leading cause of death and disability in industrialized nations, because it is the underlying problem for a number of illnesses including coronary heart disease, stroke, renal artery stenosis, and peripheral arterial disease. It is a progressive, chronic inflammatory disease resulting in the thickening of arterial walls as a result of the accumulation of cells, cell debris, and fatty compounds such as cholesterol, called an atherosclerotic plaque. The inflammation is initiated by the accumulation and oxidation of low-density lipoprotein (LDL), a carrier of cholesterol, in the blood vessel wall. The denaturation of the LDL stimulates the infiltration of monocytes from the blood, which differentiate into macrophages in the intima. The macrophages ingest the denatured LDL and its associated cholesterol to form droplet-filled cells called foam cells. The foam cells eventually die, propagating the inflammatory process (Hansson and Libby, 2006; Libby, 2002).

In the treatment and prevention of atherosclerosis, one approach is to limit the intracellular accumulation of cholesterol that adversely influences the viability of macrophages. The pathogenesis of atherosclerosis has similarities in the process of acute tissue injury and the consequent acute inflammatory process. During acute tissue injury, macrophages also become foam cells and a cholesterol elimination mechanism is necessary (Fantone and Ward, 1994). This is provided in part by serum amyloid A, which promotes cholesterol expulsion from foam cells and binds it and transports it to the liver (Tam et al., 2002). Based on this mechanism, a peptide fragment of serum amyloid A called MFFD is being examined as a possible treatment for atherosclerosis. MFFD is a water-soluble, 19-mer peptide with the sequence ADQAANEWGRSGKDPNHFR, and initial studies in mice have shown that it effectively causes the expulsion of cholesterol from foam cells in mice (Tam et al., 2005).

The long-term objective of this work is to formulate a biodegradable delivery system capable of chronic therapy of MFFD via subcutaneous implantation. The delivery platform being examined is comprised of a photocrosslinkable elastomer network based on *star*-poly(ε-caprolactone-*co*-D,L-lactide) (Amsden et al., 2004). This approach was chosen because photocrosslinking this polymer pro-

* Corresponding author. Tel.: +1 613 533 3093.
E-mail address: brian.amsden@chee.queensu.ca (B. Amsden).

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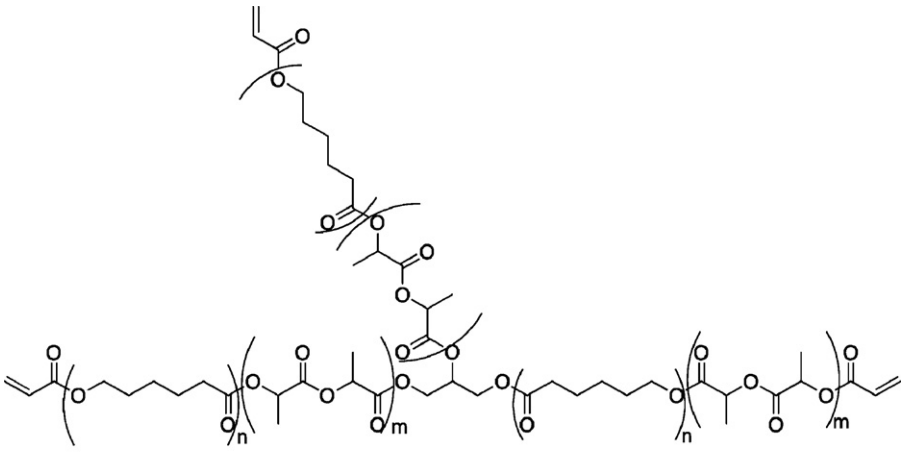
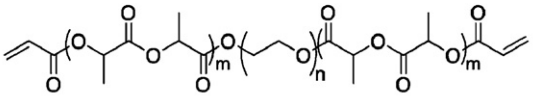


ω,ω',ω''-triacrylate *star*-poly(ε-caprolactone-*co*-D,L-lactide) (ASCP)



α,ω-diacrylate oligo(D,L-lactide)-*b*-poly(ethylene glycol)-*b*-oligo(D,L-lactide) (DLPEGDLDA)

Fig. 1. Chemical structures of the prepolymers used in the preparation of the network formulations.

vides a rapid means of entrapping solid protein or peptide particles without denaturation (Gu et al., 2007a,b; Amsden et al., 2008), release of the peptide can be controlled by the composition of the polymer, the polymer degrades via hydrolysis, and the polymer and its degradation products are well tolerated *in vivo* (Chapanian et al., in press). The degradation rate of the polymer is dependent on the molecular weight of the prepolymer used to form the network, and increases as the prepolymer molecular weight increases. However, the degradation times are relatively long, being on the order of 26–40 weeks (Chapanian et al., in press). The aim of this work was to examine the ability of copolymerization of a degradable poly(ethylene glycol) copolymer into the network to enhance the degradation rate while providing a prolonged release of MFFD. To accomplish this aim, 1000 and 4000 Da α,ω-diacrylate *oligo*(D,L-lactide)-*b*-poly(ethylene glycol)-*b*-*oligo*(D,L-lactide) (DLPEGDLDA) was copolymerized with 2700 and 5000 Da ω,ω,ω-triacrylate *star*-poly(ε-caprolactone-*co*-D,L-lactide) using UV irradiation. The structures of these prepolymers can be seen in Fig. 1. The *in vitro* degradation of the networks formed was assessed in phosphate buffered saline, along with the *in vitro* release of MFFD from the network.

2. Methods

D,L-Lactide (99%) was obtained from Purac (The Netherlands) and used as received, while ε-caprolactone was obtained from Lancaster (Canada), dried over CaH_2 (Aldrich, Canada) and distilled under vacuum in a nitrogen atmosphere. Other chemicals used were stannous 2-ethylhexanoate, glycerol, acryloyl chloride, triethylamine, 1000 and 4000 Da poly(ethylene glycol) diol (PEG), 4-dimethylaminopyridine, and 2,2-dimethoxy-2-phenylacetophenone, which were all obtained from Aldrich, Canada. Anhydrous dichloromethane and ethyl acetate were obtained from Fisher, Canada.

2.1. Polymer synthesis

An example of the procedure used to prepare the *oligo*(D,L-lactide)-*b*-poly(ethylene glycol)-*b*-*oligo*(D,L-lactide) (DLPEGDL) is as follows. 12.55 g of PEG were added to a flame-dried ampoule and dried for 12 h at 100 °C under vacuum to remove traces of water. The PEG was cooled to room temperature under vacuum and 4.25 g of D,L-lactide were added to the ampoule. The ampoule was then placed in the oven at 140 °C until the PEG and the D,L-lactide had both melted. The ampoule was removed from the oven, 0.003 g stannous 2-ethylhexanoate was added to the melt and the mixture was vortexed under vacuum. The ampoule was then flame-sealed and placed in the oven for 24 h at 140 °C. When the polymerization time had elapsed, the polymer was cooled to room temperature, and purified by precipitation from dichloromethane into diethyl ether cooled in a bath of methanol and dry ice. The precipitate was then filtered and placed under vacuum at room temperature for 3 days to remove solvents. The DLPEGDL was stored under vacuum until further use. The termini of the DLPEGDL were functionalized using acryloyl chloride in anhydrous dichloromethane containing triethylamine as an HCl scavenger and 4-dimethylaminopyridine as a catalyst, at room temperature under nitrogen for 48 h (Sawhney et al., 1993). Before acrylation, the DLPEGDL was dried under vacuum at 100 °C for 12 h to remove trace amounts of water or solvents. The acrylation reaction was carried in dried and distilled dichloromethane with triethylamine as an HCl scavenger, and the catalyst 4-dimethylaminopyridine, at room temperature under nitrogen for 48 h. A 1:1.2 molar equivalent of acryloyl chloride, and a 1:1 molar equivalent of triethylamine, to terminal hydroxyl were used, respectively. The final solution was dried under vacuum and redissolved in ethyl acetate. The precipitated HCl-triethylamine salt was removed by filtration. The ethyl acetate was dried from the filtrate and the resulting polymer was resolubilized in dichloromethane. The solution was then precipitated in excess diethyl ether that was cooled in a bath of methanol and

dry ice. The precipitate was filtered and placed under vacuum at room temperature for 3 days to remove solvents. The resulting α,ω-diacrylate DLPEGDL (DLPEGDLDA) was stored under at −20 °C until required. The degree of acrylation (*i.e.* conversion of the terminal hydroxyl groups) was measured from end-group analysis using ^{1}H NMR, as described in Amsden et al. (2006).

The photocrosslinkable *star*-poly(ε-caprolactone-*co*-D,L-lactide) was prepared as described previously (Amsden et al., 2004, 2006). Briefly, 50:50 molar ratio copolymers were prepared of molecular weights of 2700 and 5000 Da by melt ring-opening polymerization of ε-caprolactone and D,L-lactide at 140 °C for 24 h initiated by glycerol and catalyzed by stannous 2-ethylhexanoate. This process yielded a 3-armed star copolymer terminated in hydroxyl groups. The star copolymer termini were functionalized in the same manner as the DLPEGDL. Purification from dichloromethane into cold methanol yielded an acrylated star copolymer (ASCP). ^{1}H NMR analysis of the prepolymers was conducted in DMSO-d_6 using a Bruker Avance 400 MHz spectrometer.

To prepare elastomer matrices, 2700 and 5000 Da ASCP and 10 or 30 (w/w%) of DLPEGDLDA were solubilized in ethyl acetate (1:1, w:v) containing 1.5 (w/w%) of photoinitiator 2,2-dimethoxy-2-phenyl-acetophenone. The solution was then poured into 1.5 mm diameter glass tubing sealed at one end, and then capped with a septum. The prepolymers were crosslinked in the tubing by exposure to 320–380 nm ultraviolet light (EXFO Lite, Canada) at 10 mW/cm^2 for 5 min. The resulting polymer rods were dried under vacuum for 24 h, removed from their glass cylinders, cut to a length of 1.5 cm and characterized. Thermal analysis was conducted using a TA Instruments Q100 DSC. Samples were run using a heating, cooling, heating cycle as follows: ambient to −80 °C, held 10 min, heated to 120 °C, held 10 min, cooled to −80 °C, held 10 min, then heated to 120 °C. The rate of heating/cooling was 5 °C/min. The sol content of the networks was measured by weighing the dried elastomer, followed by three sequential immersions of the rods in dichloromethane for 12 h each and 24 h drying under vacuum and then re-weighing the elastomer sample.

2.2. Device preparation

MFFD was used as received from Atherochem Inc., Ottawa, Canada. MFFD loaded cylinders were prepared by first dispersing the MFFD as solid particles in a solution of ASCP and DLPEGDLDA dissolved in ethyl acetate. In this solution was also dissolved 0.015 mg 2,2-dimethoxy-2-phenylacetophenone (UV photoinitiator) per gram ASCP. The MFFD particles were suspended by agitation using a vortexer, and the suspension quickly poured into glass tubing sealed at one end. The tube was placed into a holder and rotated horizontally at 40 rpm under the UV lamp at an irradiation intensity of 10 mW/cm^2 for 5 min. One end of the tube was then opened to allow for solvent evaporation, followed by removal of the polymer/MFFD device from the glass. Cylinders of 1 cm length were cut from these master cylinders, and then placed in 20 mm Hg vacuum for 48 h to remove residual solvent prior to being used in subsequent release experiments.

2.3. Release studies

The MFFD loaded cylinders were placed in 2 ml poly(propylene) vials containing 1 ml pH 7.4 phosphate buffered saline containing 0.2 (w/v%) bovine serum albumin. The vials were placed on a rotary shaker maintained at 37 °C in an incubator oven. At each sampling period, the release medium was removed and replaced with fresh buffer. The MFFD concentration in the release media was determined using reverse-phase high performance liquid chromatography with a Waters Symmetry 5 μm C18 4.6 mm × 300 mm column. Mobile phase A consisted of 10% acetonitrile and 0.1% trifluoroacetic anhydride in water while mobile phase B was 80% acetonitrile and 0.1% trifluoroacetic anhydride in water. The column was held at a flow of 0.8 ml/min and equilibrated with 10% B. The method consisted of an isocratic hold at 10% B for 5 min, 21% B for 5 min, an isocratic hold at 21% B for 20 min, 60% B for 15 min, 80% B for 1 min and an isocratic hold at 80% B in 5 min. MFFD was detected using UV absorbance at 220 nm. All absorbance readings were corrected by the absorbance of media from control cylinders released under the same conditions, but containing no MFFD. Possible adsorption of MFFD to vessel walls was tested and rejected based on measurements of standard solutions of concentrations within the range of the release study incubated with poly(propylene) and the HPLC glass vials.

2.4. Network degradation studies

MFFD-free cylinders were prepared in the same fashion as described above. The initial mass and dimensions of the cylinders were recorded. The cylinders were immersed in 4 ml pH 7.4 phosphate buffered saline maintained at 37 °C in 5 ml glass vials. The buffer was replaced weekly. At given time points, the cylinders were removed, wiped dry with Kim Wipes, their dimensions recorded using calipers, and weighed. Three cylinders were also then dried in a vacuum oven for 48 h in the presence of dessicant, and weighed dry. The sol content of the dried samples was assessed as described above.

2.5. Statistics

All experiments were performed in triplicate, with the data points in the figures representing the average, and the error bars one standard deviation from the average. Pair-wise comparisons were assessed using one-way ANOVA with a Tukey post hoc analysis. Paired comparisons were considered significantly different for $p < 0.05$.

3. Results

3.1. Prepolymer characterization

Fig. 2 shows the NMR spectra of DLPEGDL and DLPEGDLDA along with peak assignments, while the NMR spectrum of the 5000 Da ASCP is given in Fig. 3. The spectra indicate the high degree of acrylation achieved (>95%), as demonstrated by the proton peak corresponding to the terminal hydroxyl groups at 5.48 ppm, visible in the DLPEGDL spectrum in Fig. 2A, but virtually gone in Fig. 2B. As noted previously, the *star*-poly(ε-caprolactone-*co*-D,L-lactide) has two peaks corresponding to terminal hydroxyl groups; at $\delta = 4.33$ ppm for hydroxyl groups on the terminal caproyl units and 5.48 ppm for hydroxyl groups on terminal lactyl units (Amsden et al., 2006). These peaks are also virtually absent in Fig. 3.

The peak assignments were used to calculate the ε-caprolactone to D,L-lactide monomer ratio of the ASCP, the degree of polymerization (DP) of the lactide at each end of the DLPEGDL, as well as the number average molecular weight, Mn, of the prepolymers from end group analysis. As indicated in Table 1, the Mn and the monomer composition of the ASCP were very close to the target values, as have been found previously (Amsden et al., 2006). For the DLPEGDLDA (Table 2), 4 mol of D,L-lactide were present in the polymerization mixture per mole hydroxyl, however, the DP of the product was approximately 6. This higher-than-targeted value is attributed to loss of lower molecular weight fractions during the purification stages.

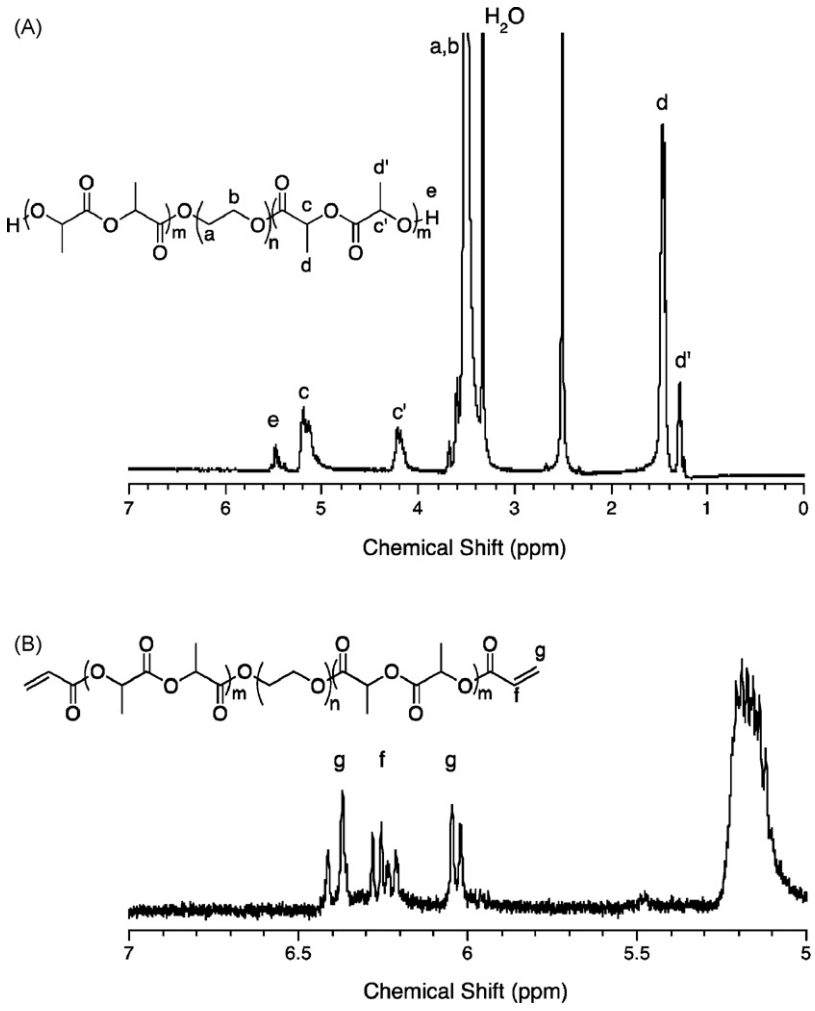


Fig. 2. ^{1}H NMR spectra of (A) DLPEGDL and (B) DLPEGDLDA along with peak assignments.

3.2. Properties of the photocrosslinked networks

Four different combinations of ASCP and DLPEGDLDA were copolymerized to obtain a range of properties indicative of the possible properties of the resulting networks (Table 3). In the subsequent discussion, the sample networks are abbreviated as ASCP Mn:PEG Mn:DLPEGDLDA%; *e.g.* 2.7:4:30% refers to the network prepared from copolymerizing a 2.7 kDa ASCP with 30% DLPEGDLDA

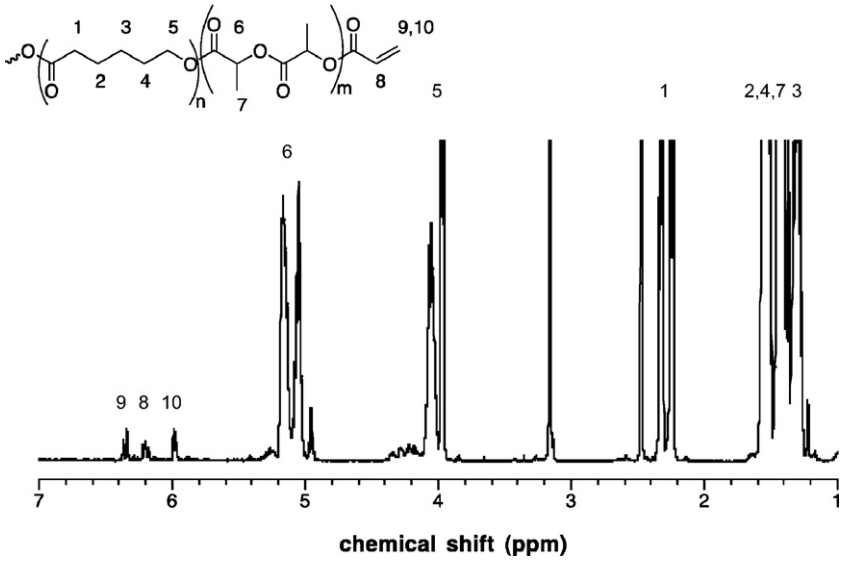


Fig. 3. ^{1}H NMR spectrum of the 5000 Da ASCP along with peak assignments.

Table 1
Properties of the acrylated star copolymer (ASCP) prepolymers.

Target Mn (Da)	Mn (NMR) (Da)	CL:DL (mol:mol)	DA (%)
2700	2605	49:51	97
5000	5025	51:49	96

Mn = number average molecular weight; CL = ε-caprolactone; DL = D,L-lactide; DA = degree of acrylation.

Table 2
Properties of the DLPEGDLDA prepolymers.

PEG Mn (Da)	Mn (NMR) (Da)	DP	DA (%)
1000	2716	5.8	99
4000	5565	6.0	99

Mn = number average molecular weight; DP = degree of polymerization of D,L-lactide; DA = degree of acrylation.

prepared using 4 kDa PEG diol. The sol contents of all the networks were less than 5% and they all had glass transition temperatures (T_g) below 0 °C, ensuring that they would be flexible at body temperature. The networks containing 30 (w/w%) DLPEGDLDA prepared from 4 kDa PEG diol (2.7:4:30% and 5:4:30%) exhibited a very small melting endotherm at 49.5 and 45 °C, respectively (Table 3) during the first heating cycle. This endotherm disappeared during the second heating cycle. For these same two networks, the T_g decreased as the ASCP molecular weight increased, indicative of an increase in crosslink density as the ASCP molecular weight decreases. The networks containing 1000 Da PEG of 30 (w/w%) (5:1:30%) were, by contrast, amorphous, as were the 5:4:10% networks. Upon immersion in water, the glass transition temperature of the networks decreased (Table 3), due to water absorption. The absorbed water plasticizes the networks, increasing chain mobility.

3.3. *In vitro degradation*

The change in dry mass of the networks immersed in PBS buffer at pH 7.4 is given in Fig. 4A. For comparison, the degradation of networks prepared only from ASCP 2700 (2.7:0:0%), previously reported (Amsden et al., 2004), are also included. For all the networks containing PEG, there was little observable mass loss over the first 6 weeks, followed by an onset of relatively rapid mass loss at 10 weeks. For the 2.7:0:0% networks, the onset of measurable weight loss was not observed until 12 weeks. The PEG-containing networks were completely degraded by 17 weeks. There was not a significant influence of the molecular weight of the PEG used in preparing the DLPEGDLDA on the overall degradation rate, as can be observed from a comparison of the 5:4:30% and 5:1:30% networks. These networks retained 82 ± 7% and 71 ± 3% of their initial mass at week 10, respectively, and 23 ± 9% and 28 ± 11% of their initial mass at week 12, respectively. The rate of mass loss was determined primarily by the molecular weight of the ASCP used in preparing the network. For example, the networks containing 30% of the DLPEGDLDA prepared from the 4000 Da PEG, but with different ASCP molecular weight (5:4:30% and 2.7:4:30%), retained 82 ± 7% and 88 ± 1% of their initial dry masses at week 10, respectively. However, by week 12, the same networks retained 23 ± 9% and 52 ± 8% of their initial mass, respectively. These values were significantly different ($p < 0.01$).

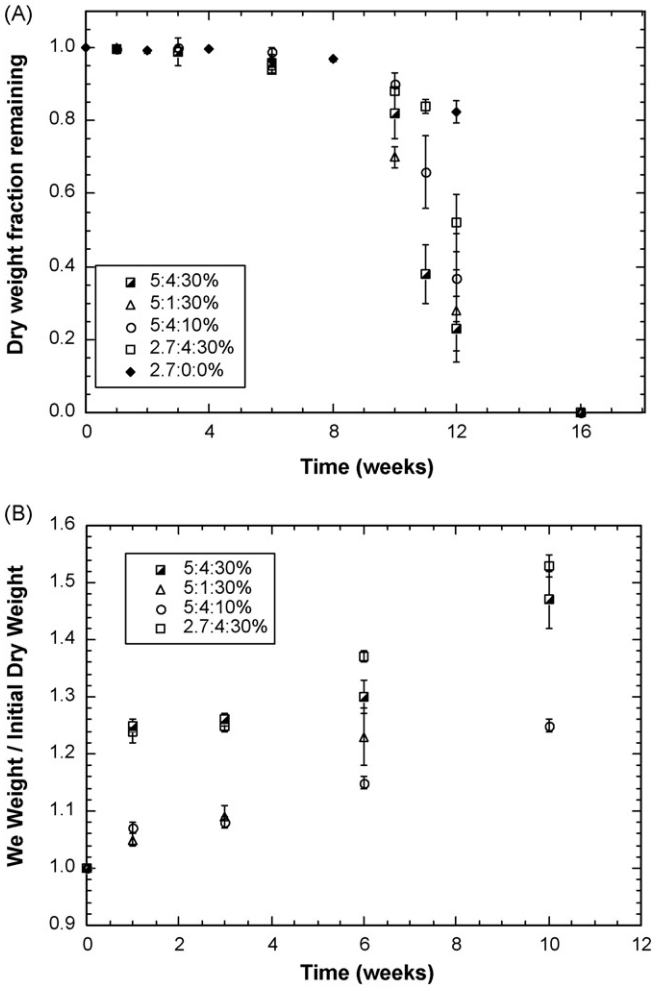


Fig. 4. Change in MFFD-free network properties during *in vitro* degradation in pH 7.4 phosphate buffered saline. (A) Dry weight change with time. The data for the degradation of the 2700 Da network without DLPEGDLDA (27:0:0%) was taken from (Amsden et al., 2004). (B) Wet weight change with time.

As has been observed for networks prepared using the same ASCP but with α,ω-diacrylate PEG (Amsden et al., 2008), there was an initial increase in wet mass during *in vitro* degradation as the networks absorbed water. After this initial absorption, the wet weight of the networks remained unchanged over the next 2 weeks (Fig. 4B). By 6 weeks, the wet weight began to increase, and by 10 weeks the networks had swelled to an extent that was significantly greater than the volume at 2 and 3 weeks ($p < 0.01$); the 5:4:30% networks had a swelling increase of 17%, the 5:4:10% a swelling increase of 16%, the 2.7:4:10% a swelling increase of 22%, and the 5:1:30% a swelling increase of 17%. Beyond 10 weeks, the networks had no mechanical stability and could not be handled to have their weight measured. The degree of swelling of the different networks

Table 3
Thermal properties of the photocrosslinked networks.

ASCP Mn (Da)	PEG Mn (Da)	wt% DLPEGDLDA	Abbreviation	T_g (°C)	T_m (°C)	ΔH (J/g)	T_g wet[b] (°C)
2700	4000	30	2.7:4:30%	−12.5	49.5[a]	0.4[a]	−15
5000	4000	30	5:4:30%	−24	45[a]	0.4[a]	−26
5000	4000	10	5:4:10%	−13	–	–	−10
5000	1000	30	5:1:30%	−8.1	–	–	−11

[a] First heating cycle only.
[b] Measured after 1 week immersion in PBS.

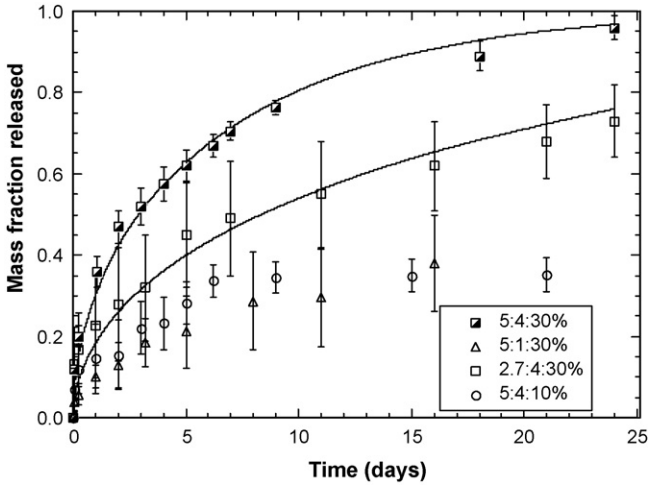


Fig. 5. Influence of network polymer composition on the *in vitro* release of MFFD. The solid lines represent fits of the solution to Fick's 2nd law expression for diffusion from a cylinder (Eq. (1)) to the data indicated.

was driven principally by the amount and molecular weight of the PEG incorporated. The molecular weight of the ASCP used in preparing the networks had negligible effect, while increasing the PEG content at a constant PEG molecular weight, and increasing the PEG molecular weight at a constant PEG content, each increased the degree of swelling; the 5:4:30 and 2.7:4:30% networks each swelled to the same extent over the first 3 weeks (approximately 125% by weight) as did the 5:4:10 and 5:1:30% networks (approximately 108% by weight).

3.4. MFFD release

The rate and extent of MFFD release from the networks over the first 24 days is shown in Fig. 5. The amount and duration of MFFD released was in general dependent on the initial swelling extent of the networks over the first 3 weeks, with release from the more swollen networks being more complete and more prolonged. The networks that swelled only about 8–10 (w/w%) (5:1:30 and 5:4:10%) released very little (~35%) of the incorporated peptide by 10 days, and the amount released did not increase appreciably after this time. Subsequent release would require degradation of the polymer network. By contrast, the networks that swelled approximately 35 (w/w%), (5:40:30 and 2.7:4:30%) released about 95 and 73% of the initially loaded peptide by 24 days, respectively. For these latter networks, the following equation, which is a solution to Fick's second law of diffusion for release from a cylinder into an infinite sink (Crank, 1975), was fit to the data:

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{4}{r^2\alpha_n^2} \exp\left(\frac{-D\alpha_n^2 t}{r^2}\right) \quad (1)$$

wherein α_n's are the positive roots of $J_0(r\alpha_n) = 0$, J_0 is a Bessel function of the first kind of order zero), M_t is the mass released by time t, M_∞ is the total mass initially loaded, and r is the radius of the cylinder. Eq. (1) was fit to the data by considering the diffusion coefficient, D, to be an adjustable parameter. The solid lines in Fig. 5 represent the least-squares regression to the data. The close agreement between the fitted curve and the data indicate that the peptide was released primarily via diffusion. The diffusion coefficients were calculated to be 8.33 ± 0.3 (10^{-8}) cm^2/s and 1.17 ± 0.4 (10^{-8}) cm^2/s for the 5:4:30 and 2.4:4:30% networks, respectively.

The possibility of structural changes to the MFFD as a result of the photocrosslinking conditions was tested by examining the HPLC elution curves of released MFFD over the initial and latter time points. As shown in Fig. 6A, there was no change in the time at which MFFD eluted from the column at the first time point of 1 h, and there was no evidence of other peaks. This result was taken to mean that the MFFD retained its original structure during device fabrication. A comparison of the elution curves for MFFD released from the 5:4:30 and 2.7:4:30% cylinders at 24 days also do not show any peaks other than those of the peptide eluting at the same time as the MFFD standard (Fig. 6B). This result was taken as evidence that the peptide did not undergo degradation during the release period.

4. Discussion

PEG is known to undergo slow crystallization when blended with amorphous poly(lactide) (Hu et al., 2003) and poly(ε-caprolactone) is immiscible with PEG at temperatures lower than about 40 °C (Chuang et al., 2005). Moreover, previous findings by our group showed that when α,ω-diacrylate PEG was copolymerized with ASCP, phase-separated and crystallized PEG regions were observable throughout the bulk that produced distinct melting endotherms at α,ω-diacrylate PEG levels as low as 10 (w/w%) in the network (Amsden et al., 2008). However, copolymerizing α,ω-diacrylate *oligo*(D,L-lactide)-*b*-PEG-*b*-*oligo*(D,L-lactide) with the ASCP resulted in networks wherein the triblock PEG copolymer was almost completely molecularly dispersed throughout, as indicated by the very small melting endotherm associated with PEG regions found in the first heating cycle. The disappearance of this endotherm during the second heating cycle is consistent with the slow crystallization event for PEG reported by Hu et al. (2003). The incorporation of the D,L-lactide onto the PEG diol termini thus improved the miscibility of the PEG within the ASCP. This effect was more pronounced when the lower molecular weight PEG triblock copolymers were incorporated.

It is interesting to compare the *in vitro* degradation behavior of networks prepared previously that did not contain PEG (Amsden et al., 2004) with the networks of this study. Fig. 4A also shows the mass change with time of networks prepared from 2700 Da ASCP of the same monomer content as prepared in this work (2.7:0:0%) alongside that of the 2.7:4:30% networks. In each case, there was little observable mass loss over the first 6 weeks, and the onset of significant mass loss occurred at approximately the same time (between 8 and 10 weeks). Beyond this time, however, the DLPEGDLDA-containing networks exhibited increased mass loss; networks prepared solely of 2700 Da ASCP of the same monomer content as prepared in this work lost only 18% of their initial mass by 12 weeks, whereas the incorporation of 30 (w/w%) DLPEGDLDA into 2700 Da ASCP networks resulted in a mass loss at the same time frame of 48%. Both degradation profiles are consistent with a bulk erosion degradation mechanism. As shown previously in the *in vitro* degradation of networks that did not contain PEG, wherein the degradation rate increased as the network crosslink density increased (*i.e.* as Mn of ASCP decreased) (Chapanian et al., in press), increasing ASCP molecular weight significantly influenced the degradation rate. These results are in contrast to the previous results obtained using PEGDA incorporation into ASCP networks, wherein the degradation rate of the networks was not influenced by the presence of the PEG in the network, which was attributed to the very slow degradation of the acrylate ester on the PEG chain ends (Amsden et al., 2008). Thus, the hydrolysis rate of the ester linkages in each polymer network is apparently the same initially and the mass loss of the networks is influenced primarily by degradation of the poly(ε-caprolactone-*co*-D,L-lactide) backbone, which is present in highest concentration. However, the enhanced swelling due to the presence of DLPEGDL likely facilitates the loss of water-soluble degradation products from the network and the degradable

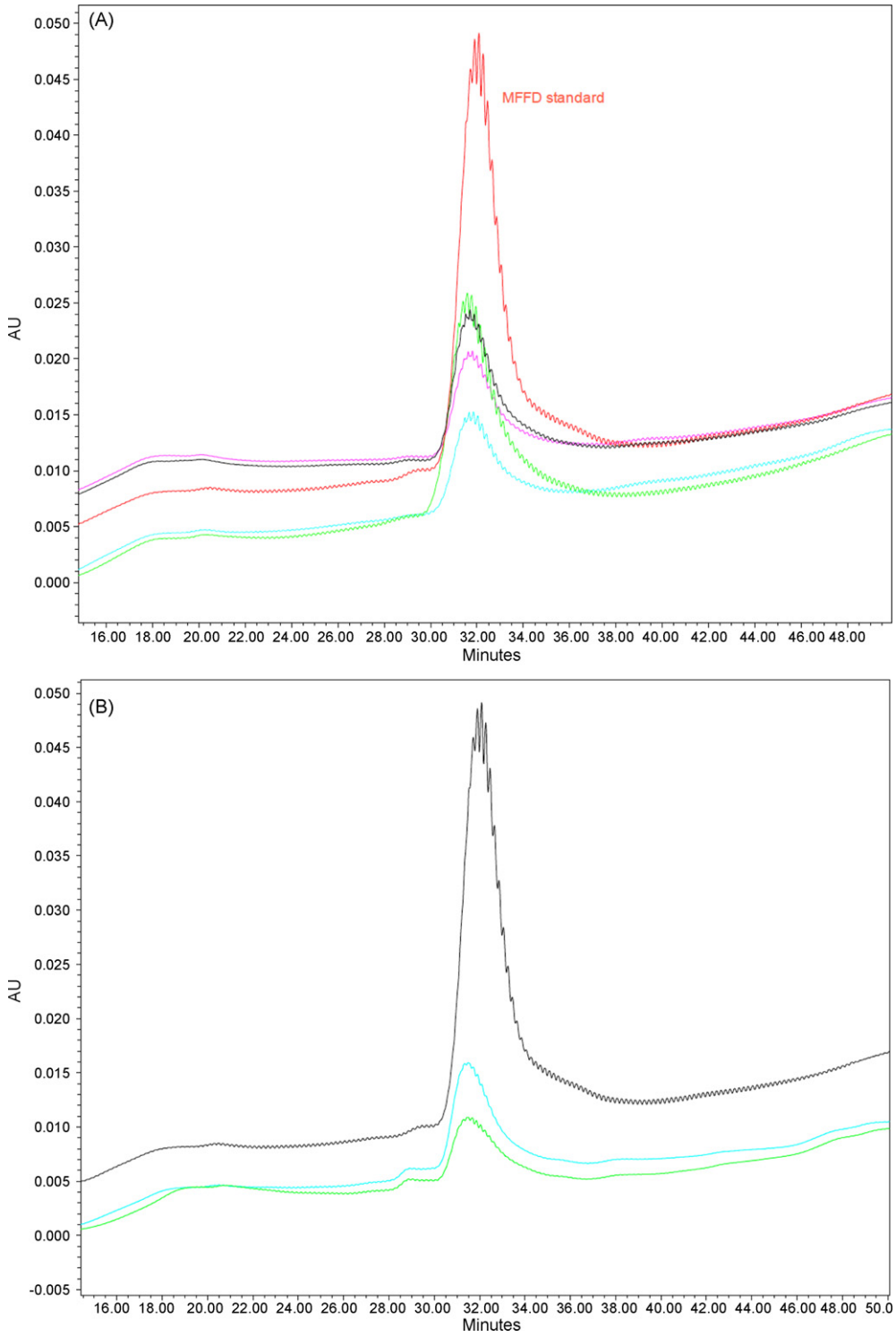


Fig. 6. HPLC elution profiles of the MFFD in the release medium versus a 28 mg/ml MFFD standard. (A) Comparison of the elution profiles at the first sampling time of 1 h. The top curve is that of the MFFD standard, while the remaining curves are for MFFD released from: 5:4:30% (light blue), 5:4:10% (purple), 2.7:4:30% (black), and 5:1:30% (dark blue). (B) Comparison of the elution profiles at 24 days. The top curve is that of the MFFD standard, while the remaining curves are for MFFD released from 5:4:30% (light blue) and 2.7:4:30% (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

linkage at the DLPEGDL chain ends contributes significantly to the overall rate of mass loss. The accumulation of these water-soluble products within the networks contributes to the observed swelling that occurs after 6 weeks. These degradation products draw water into the network as a result of osmotic pressure effects (Brunner et al., 1999), and the increased water content then enhances the degradation rate of the network.

As the mass loss of the networks remained essentially unchanged over the first 3–4 weeks, it can be concluded that the release of MFFD from the networks over the time frame examined was relatively unaffected by the degradation of the networks. For the more highly swollen networks, release was diffusional, as indicated by the close agreement with the fit of Eq. (1) to the data. The MFFD was released by dissolution in water within the poly-

mer phase followed by transport through water-swollen regions in the network, generated by the presence of the PEG. The degree of swelling of the network influenced the release rate, and was controlled by both the molecular weight and amount of PEG incorporated while being insensitive to the molecular weight of the ASCP. However, the two networks containing 30% of DLPEGDLDA made with 4 kDa PEG swelled to the same degree, yet the release rates differed. For transport through gels, solute passage is controlled by the mesh size of the gel (Lustig and Peppas, 1988; Amsden, 1998, 1999). For uncharged polymer gels, the mesh size, ξ, can be expressed from scaling relationships as (Schaefer, 1984):

$$\xi \sim \phi^{-0.75} C^{-0.25} (1 - 2\chi)^{-0.25} \quad (2)$$

In Eq. (2), ϕ is the volume fraction of polymer in the gel, C is the polymer characteristic ratio and χ is the polymer–water interaction parameter. The volume fraction of polymer is constant in this situation, leaving only the characteristic ratio and/or the polymer–water interaction parameter as the factors influencing the mesh size. As swelling is driven solely by the nature of the PEG used, and the PEG was the same in each case, it is reasonable that the difference in the mesh size is due to the differences in the stiffness of the polymer chains in the water-swollen regions. This explanation is supported by the glass transition temperatures of swollen 5:4:30 and 2.7:4:30% networks measured after 1 week immersion in buffer, and found to be −26 and −15 °C, respectively (Table 3). The lower glass transition temperature of the 5:4:30% network indicates a greater degree of plasticization due to water absorption, and thus more flexible polymer chains and a lower C value and therefore a greater mesh size. The larger mesh size results in less restriction to diffusional transport within the gel and hence faster release.

5. Conclusions

A relatively rapidly degrading network formulation has been prepared and shown to effectively control the release of a water-soluble, 19 mer, therapeutic peptide, MFFD, which is being investigated for the treatment of atherosclerosis. This network consists of a terminally acrylated *star*-poly(ε-caprolactone-*co*-D,L-lactide) (ASCP) co-photocrosslinked with α,ω-diacrylate *oligo*(D,L-lactide)-*b*-PEG-*b*-*oligo*(D,L-lactide). The network degrades *in vitro* in a bulk degradation fashion at a rate controlled by the molecular weight of the ASCP used, and is complete within 16 weeks. Drug release from these networks can be controlled through selection of the molecular weight of the PEG used in preparing the α,ω-diacrylate *oligo*(D,L-lactide)-*b*-PEG-*b*-*oligo*(D,L-lactide), which controls the degree of swelling of the network, along with the molecular weight of the ASCP, which influences the mesh size of the swollen network. The peptide is unaffected by the UV initiated crosslinking procedure and remains stable in the network during release for those cylinders exhibiting nearly complete release.

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