

Synthesis and characterization of photocrosslinkable, degradable poly(vinyl alcohol)-based tissue engineering scaffolds

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Abstract

Hydrogels have many advantages that make them prime candidates for tissue engineering applications: high water content, tissue-like elasticity, and relative biocompatibility. We aim to tissue engineer heart valves using a hydrogel scaffold based on poly(vinyl alcohol) (PVA), and the design parameters for a suitable tissue engineering scaffold are quite stringent. In this research, we develop degradable and photocrosslinkable poly(lactic acid)-*g*-PVA multifunctional macromers that can be reacted in solution to form degradable networks. The mass loss profiles and bulk properties of the resulting scaffolds are easily tailored by modifying the structure of the starting macromers. Specifically, altering the number of lactide repeat units per crosslinking side chain, percent substitution, molecular weight of PVA backbone, and macromer solution concentration, the rate of mass loss from these degradable networks is controlled. In addition, by increasing the network's hydrophobicity, valve interstitial cell adhesion is improved. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Over the past decade, numerous synthetic and natural degradable biomaterials have been developed and investigated for drug delivery applications and/or as scaffolds for engineering of various organs and tissues. Poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers (PLGA) were among the first degradable polymers to be used to regenerate tissues of defined sizes and shapes [1]. Polyurethanes with either hydrolyzable phosphoesters [2] or lysine methyl esters [3] and bacterially derived polyhydroxyalkanoates [4,5] are important degradable elastomeric materials that have great potential for uses in biomedical applications. Degradable polyanhydrides have been investigated for orthopedic applications [6] and to treat brain cancer [7]. Degradable poly(ether-anhydride) networks have recently been developed and provide a variety of properties ranging from hydrophilic hydrogels to hydrophobic

networks [8]. Numerous degradable hydrogels have been investigated for drug delivery and tissue engineering applications, including dextran-based hydrogels for controlled drug delivery [9,10], glycosaminoglycan-augmented polysaccharide hydrogels for tissue engineering articular cartilage [11], and alkoxide-crosslinked hydrogels as degradable superabsorbents [12].

Our research group is particularly interested in approaches to tissue engineering heart valves, and the requirements for a suitable scaffold are quite stringent. Due to the elastic nature of the heart valve, the polymer scaffold must provide not only the appropriate flexibility and elasticity found in the native valve, but also adequate mechanical strength. To incorporate sufficient mechanical strength and flexibility, we are interested in designing poly(vinyl alcohol) (PVA)-based hydrogel scaffolds for valve tissue engineering. Hydrogels, in general, have a high water content and tissue-like elasticity. Furthermore, the abundant hydroxyl groups on PVA can be readily modified to attach growth factors, adhesion proteins, or other molecules of biological importance. Finally, PVA is bio-inert and, therefore, relatively biocompatible [13].

Despite the potential advantages of PVA scaffolds, the ability of cells to attach to hydrogels is limited. We

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have previously shown [14] that attaching important cell adhesion proteins, such as fibronectin, onto the surface of glutaraldehyde-crosslinked PVA hydrogels promotes the attachment of cells. The inability of cells to attach to hydrogels, in general, relates to their hydrophilicity, leading to minimal adsorption of cell adhesion proteins on the gel surface. By covalently linking fibronectin onto PVA hydrogel surfaces, fibroblast [14] and valve interstitial cell (VIC) (unpublished data) attachment, proliferation, and migration were dramatically improved.

Using this base scaffold chemistry, macroporous, three-dimensional fibronectin-modified PVA hydrogel scaffolds were synthesized and seeded with valve interstitial cells [15]. While this scaffold was compatible with VICs and promoted their attachment and proliferation, extracellular matrix deposition was low. We hypothesized that minimal extracellular matrix deposition was due to the high hydrophilicity of the hydrogel. Moreover, the glutaraldehyde-crosslinked PVA is non-degradable, and additional chemical modifications were needed to create degradable gels.

To test the influence of scaffold hydrophobicity on extracellular matrix deposition, hydrophobic scaffolds based on diethylene glycol and lactide repeat units (designated by 2EG10LA) were examined and compared to hydrophilic PVA scaffolds. While the poly(2-EG10LA) scaffolds allowed better cell attachment and extracellular matrix deposition, the macroscopic material is brittle and inflexible [15,16]. A valve construct made of this material would be unable to open and close properly due to its inelasticity. However, this scaffold is degradable and can be photopolymerized into a variety of shapes, which provides certain benefits.

In this paper, we aim to develop a class of cross-linkable PVA macromers that provide the benefits of both PVA and poly(2EG10LA) without the inherent disadvantages of either. Specifically, the PVA macromers should be readily processed into complex shapes, hydrolytically degradable, and possess chemical properties (i.e., hydrophobicity) that can be tailored to affect extracellular matrix production. To achieve these objectives, multifunctional, brush-like macromers were synthesized by grafting PLA side chains onto the PVA backbone. These degradable grafts were end-capped with photocrosslinkable methacrylate groups to enable facile processing of crosslinked hydrogels. The overall hydrophobicity, which is influenced by the length and number of grafts, and the final crosslinking density, which is directly related to the functionality of the macromers, control the final network properties. We hypothesize that degradable PVA networks with increased hydrophobicity will impart desirable mechanical properties and support extracellular matrix deposition. The PVA component will enable adequate elasticity, and the abundant hydroxyl groups on PVA can be used to

incorporate molecules of biological significance. The objectives of this contribution are to develop the chemistry to synthesize multifunctional PLA-graft-PVA macromers, characterize their degradation behavior as a function of the macromer chemistry, and assess their potential to serve as a scaffold for the tissue engineering of heart valves.

2. Materials and methods

2.1. HEMA-Lac

Methacrylated macromers with degradable side chains were synthesized similar to the method used by van Dijk-Wolthuis et al. [17]. In a 50-ml round bottom flask, 5.0 g hydroxyethyl methacrylate (38 mmol, Aldrich) and a desired stoichiometric amount of lactide (Polysciences) were melted at 110°C under a nitrogen atmosphere. Two grafted HEMA-Lac molecules were synthesized (see Table 1) incorporating different numbers of lactide repeat units into the chain, representing different numbers of theoretically hydrolyzable ester linkages. A trace amount of hydroquinone (Aldrich) was added to the flask to inhibit free-radical polymerization of the methacrylate groups. Once molten, stannous octoate (1:200 molar ratio to lactides) was added, and the reaction was allowed to proceed at 110°C for 1 h (see Fig. 1). No further purification was performed on the HEMA-Lac product, and NMR analysis was conducted to verify the formed product. The actual number of hydrolyzable linkages was calculated by dividing the area under the lactic acid methyl proton peak (1.4–1.6 ppm) by the hydroxyl proton peak (2.7 ppm) area.

2.2. HEMA-Lac-Suc

The HEMA-Lac reaction mixture was cooled to 50°C, and 3.85 g succinic anhydride (38 mmol, Aldrich), 25 ml pyridine (Aldrich), and 0.5 g DMAP (Fluka) were added to the reaction. The reaction was allowed to proceed for 16–18 h at 50°C. To remove unreacted succinic anhydride, glutaraldehyde-crosslinked poly(vinyl alcohol) disks (PVA-GA) were added to the mixture for 24 h at room temperature. The pendant hydroxy groups on the

Table 1
Compositions of the two different HEMA-Lac syntheses studied and the actual number of hydrolyzable ester groups per molecule, as determined by NMR

Molecule	Theoretical hydrolyzable groups	Actual hydrolyzable groups
HEMA-4Lac	4 ($n = 2$)	3.6
HEMA-8Lac	8 ($n = 4$)	7.2

PVA-GA gels readily react with the unreacted succinic anhydride, and the gels are easily separated by filtration from the reaction solution. PVA-GA disks were made by adding dilute HCl to a 15 wt% PVA (25,000 MW, Polysciences) solution in dH₂O with 2% glutaraldehyde (Sigma). Upon purification of the macromer solution using the PVA-GA disks, the disks were removed from the reaction mixture, and no further purification of the HEMA-Lac-Suc product was performed. Fig. 1 shows the addition of lactides to HEMA and the ring opening of succinic anhydride by the free hydroxyl group to convert it to an acid group. NMR analysis was used to verify conversion of the hydroxyl group to the acid group by comparing the succinic anhydride group protons (2.6–2.8 ppm) to the methacrylate protons (5.6 and 6.1 ppm). For both HEMA-4Lac and HEMA-8Lac, conversion was very efficient at >95%.

2.3. PVA-Deg

Degradable PVA macromers (PVA-Deg) were synthesized by linking HEMA-Lac-Suc chains to the hydroxyl groups of PVA. This coupling procedure was performed using the common coupling reagent dicyclohexyl carbodiimide (DCC, Fluka). The HEMA-Lac-Suc reaction mixture containing the product, pyridine, and DMAP was used directly from the previous step with no further workup or purification. An appropriate amount of PVA (6000 MW, Polysciences) was dissolved in dimethyl sulfoxide (15 wt% solution) and added to the HEMA-Lac-Suc mixture such that various theoretical percent substitutions of the hydroxyl groups on PVA with HEMA-Lac-Suc chains were obtained (see Table 3).

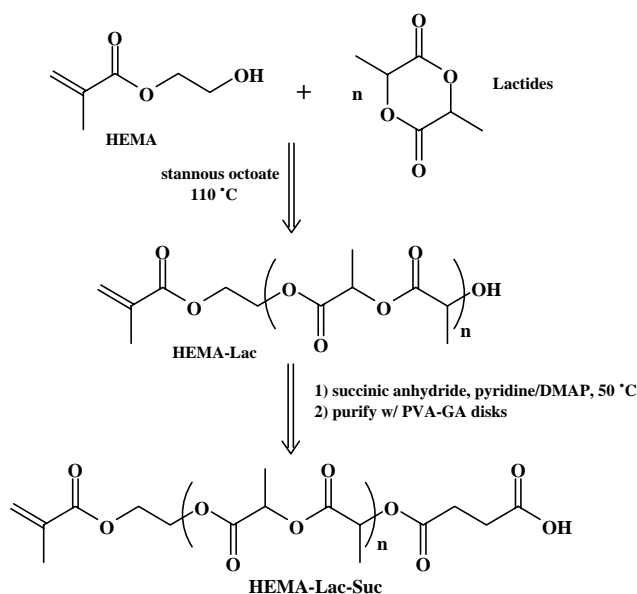


Fig. 1. Synthesis of HEMA-Lac-Suc.

DMSO was added (between 10 and 50 ml) arbitrarily such that the viscosity of all the reaction mixtures was about the same. Dicyclohexyl carbodiimide (DCC) was then added at a 1.5:1 molar ratio to the theoretical number of acid groups present in the HEMA-Lac-Suc mixture. The reaction was allowed to proceed for 24 h at room temperature. Upon coupling a hydroxyl group to an acid group, DCC is converted to dicyclohexyl urea (DCU), which precipitates out of solution. The DCU precipitate was filtered through filter paper (Whatman No.1), and the filtrate was collected and precipitated in cold ethyl ether. A small sample of the precipitate (PVA-Deg) was collected and dried in a vacuum oven overnight, and NMR was performed on the sample to calculate the actual percent substitution of the hydroxyl groups on PVA for that particular batch. The experimental percent substitution was calculated by dividing the average of the area under both methacrylate protons (5.7 and 6.0 ppm) by the sum of the average of both methacrylate protons and the hydroxyl proton (3.8 ppm).

The remainder of the precipitate was immediately placed into DMSO (usually about 1–2 times the volumetric amount of precipitate) and placed in a vacuum oven at 50 °C for several hours to remove any remaining ethyl ether. As the ethyl ether evaporated, the PVA-Deg dissolved in the DMSO. At first, the product was dried completely in a vacuum oven to remove all ethyl ether. However, it was found that the resulting dry product would not redissolve in DMSO for subsequent steps. It is believed that this insolubility was due to the formation of crystalline regions during drying that act as physical crosslinks between chains, preventing easy dissolution of the polymer. Instead, the product just swelled to a gel-like material.

When all the ethyl ether had been removed from the PVA-Deg/DMSO solution, it was cooled to room temperature. A small sample was placed in a pre-weighed 25 ml round bottom flask, weighed again with the sample, and the DMSO was evaporated using a rotary evaporator under reduced pressure and high temperature (75 °C). By measuring the final flask weight, the exact percent of PVA-Deg in solution could be calculated. Fig. 2 shows the coupling of HEMA-Lac-Suc to PVA.

2.4. PVA-Deg gel formation

A macromer solution of PVA-Deg was prepared by adding DMSO to or removing (via rotary evaporation) DMSO from the above solutions. The photoinitiator Irgacure 2959 (Ciba-Geigy) was added at 1 wt% of the entire solution, and the macromer solution was placed in a Teflon mold (disks, 1.4 cm in diameter, 2.0 mm thick) between two glass slides. The mold was placed under ultraviolet light (5.0 mW/cm²) of wavelength 365 nm for

10 min to polymerize disk-shaped gels. All gels made from the macromer solutions were made in the same sized mold. These gels were then placed into a large

excess of dH₂O for 4–5 h to remove DMSO and any sol fraction.

2.5. Determination of gel fraction

After polymerization, disks were placed in an excess of DMSO and swollen overnight to remove any polymer chains that had not been incorporated into the network (i.e., the sol fraction). Disks were then solvent-exchanged in ethanol for several hours and dried in a vacuum oven overnight to remove ethanol. Dry weights of the disks were measured, and using the initial weight fraction of polymer in the macromer solution, the gel and sol fractions were calculated.

2.6. Degradation studies

Disks of varying composition were degraded in a phosphate-buffered solution (pH 7.4) (PBS) at 37°C, and mass loss and swelling were monitored until complete dissolution. At each selected time point, three disks of each composition were removed from PBS, and the wet weights of all disks were measured. These disks

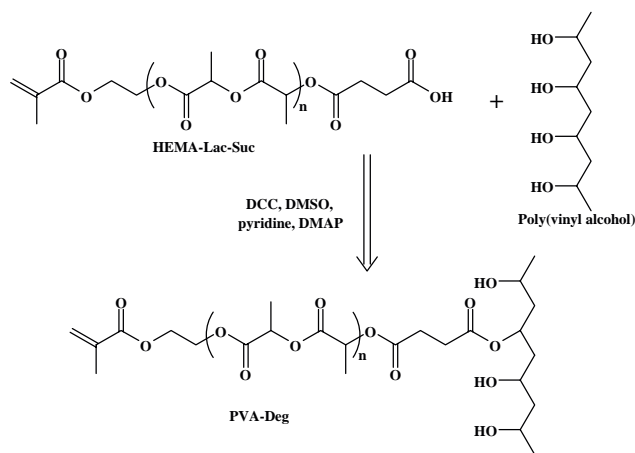


Fig. 2. Coupling reaction of HEMA-Lac-Suc to PVA to make multifunctional PVA-Deg macromers that can be chain polymerized to form degradable networks.

Table 2

Summary of macromers synthesized with respect to number of lactide repeat units per side chain, actual percent substitution (PS), and macromer solution concentration in DMSO

Macromer	HEMA-Lac no. of lactide repeat units ^a	PVA molecular weight (K)	Coupling reaction		Gel formation	
			Actual PS ^b	No. of double bonds/chain	% Macromer solution	Gel fraction
A	4	6	5	7	40	—
B	4	6	7.5	10	40	0.55
C	4	6	17	24	15	0.45
D	8	6	6	8	40	0.70
E	8	6	3	4	40	—
F	8	6	4	5	20	—

^aTheoretical; actual number of lactide repeat units were 3.6 for macromers A through C and 7.2 for macromers D through F as determined by NMR.

^bAs determined by NMR.

Table 3

Time for complete degradation of samples and initial mass swelling ratio, q , as a function of percent substitution and macromer solution concentration

Macromer	No. of lactide repeat units	Actual percent substitution (PS)	Macromer solution concentration (%)	Time for complete degradation	Initial q^a
A	4	5	40	21 days	10.0
B	4	7.5	40	> 31 days ^b	2.75
C	4	17	15	> 31 days ^c	4.0
D	8	6	40	~ 80 days	2.0
E	8	3	40	18 days	6.75
F	8	4	20	< 7 days	6.0

^aError on the mass swelling ratio was estimated to be ± 0.3 .

^b25% mass loss at 31 days.

^c30% mass loss at 31 days.

were then frozen and lyophilized overnight to remove water and weighed again to obtain the dry weight. The mass swelling ratio, q , was calculated by ratioing the wet weight to the dry weight of each disk. Finally, the percent mass loss at a particular time point was calculated by subtracting the current dry weight from the initial dry weight and normalizing by the initial dry weight. Variables such as the number of hydrolyzable ester linkages, percent substitution, and composition of the starting macromer solution (Table 2) were investigated with respect to their effects on degradation time and behavior.

2.7. Cell attachment to PVA-Deg

It was hypothesized that, by increasing the percent substitution and, therefore, the proportion of HEMA-Lac in the macromer, cell adhesion to the scaffold would be improved. Valve interstitial cells (VICs) were isolated and cultured following the method reported by Johnson et al. [18] and were seeded onto thin films of macromers B, C, and E. One day after cell seeding, photographs were taken of the cells to assess their ability to attach to the polymer.

3. Results and discussion

3.1. HEMA-Lac-Suc

The HEMA-Lac molecule contains a free hydroxyl group, which is relatively unreactive towards other chemical groups, so it was converted to an acid group using succinic anhydride. The hydroxyl group reacts with the anhydride, opening the ring and leaving a free acid group, which can easily react with the hydroxyl groups on PVA using the common coupling reagent dicyclohexyl carbodiimide (DCC). There are reactions that can be used to couple hydroxyl groups to other hydroxyl groups, but these techniques were not used because non-specific reactions between two hydroxyl groups on PVA could occur, or two HEMA-Lac molecules could be linked together, both of which are undesirable. By converting the hydroxyl group on HEMA-Lac to an acid group, a reaction that specifically linked an acid group to a hydroxyl group allowed for a much more efficient coupling of the HEMA-Lac chains to the PVA backbone with no undesirable side reactions or byproducts.

3.2. PVA-Deg

HEMA-Lac-Suc molecules were covalently linked to PVA through reaction of the acid groups on HEMA-Lac-Suc to the hydroxyl groups on PVA using DCC. The resulting ester bonds are stable, and dicyclohexyl

urea (DCU) is the byproduct. In the solvent, DMSO, DCU precipitates out of solution and can easily be filtered out of the reaction mixture. The resulting solution contained the final product, PVA-Deg, and the desired macromer solution concentration was prepared by either adding or subtracting DMSO.

3.3. PVA-Deg gel formation

Free radical chain polymerization occurs through the reactive methacrylate groups. Kinetic chains are formed when photoinitiated radicals propagate through the carbon-carbon double bonds to produce the crosslinked network. The weight fraction of the methacrylate groups in the macromer controls the concentration of kinetic chains in the gel, and the length of the kinetic chains is controlled by the relative rate of polymerization to the rate of chain terminating events. Thus, changes in the macromer concentration or concentration of initiator will influence the gel structure.

Fig. 3(i) depicts a general network structure upon polymerization of PVA-Deg. Crosslinks in the network are formed when reactive side chains from different PVA chains react together via free radical chain polymerization of the carbon-carbon double bonds (A). When reactive side chains from the same PVA molecule react with one another, cycles form (B). Cyclization does not contribute to the mechanical strength of the network and is more frequent in dilute macromer solutions. Moreover, it is likely that multiple links from the same PVA chain are incorporated into the same kinetic chain (C).

3.4. Gel fractions

The gel fractions of three of the macromers were measured. Gels made of macromer D (8 degradable units per chain, 6% substitution, 40% macromer solution) had the highest gel fraction of the three at 0.70. Macromer B (4 degradable units per chain, 7.5% substitution, 40% macromer solution), which was similar to macromer D except it had fewer repeat lactide units per chain, produced gels that were found to have gel fractions of 0.55. Finally, gels made of macromer C (4 degradable units, 17% substitution, 15% macromer solution) had gel fractions of 0.45. In general, the initial network structure and mass erosion profile will be strongly influenced by the amount of sol fraction (i.e., extractables) that is present after polymerization. The relatively large sol fraction measured in these gels is indicative of the non-idealities of multifunctional macromer polymerizations, and possibly a broad distribution of percent substitution on the PVA macromers. Furthermore, controlling the reaction conditions (e.g., macromer concentration in solution and rate of initiation) can have dramatic effects on the extractable

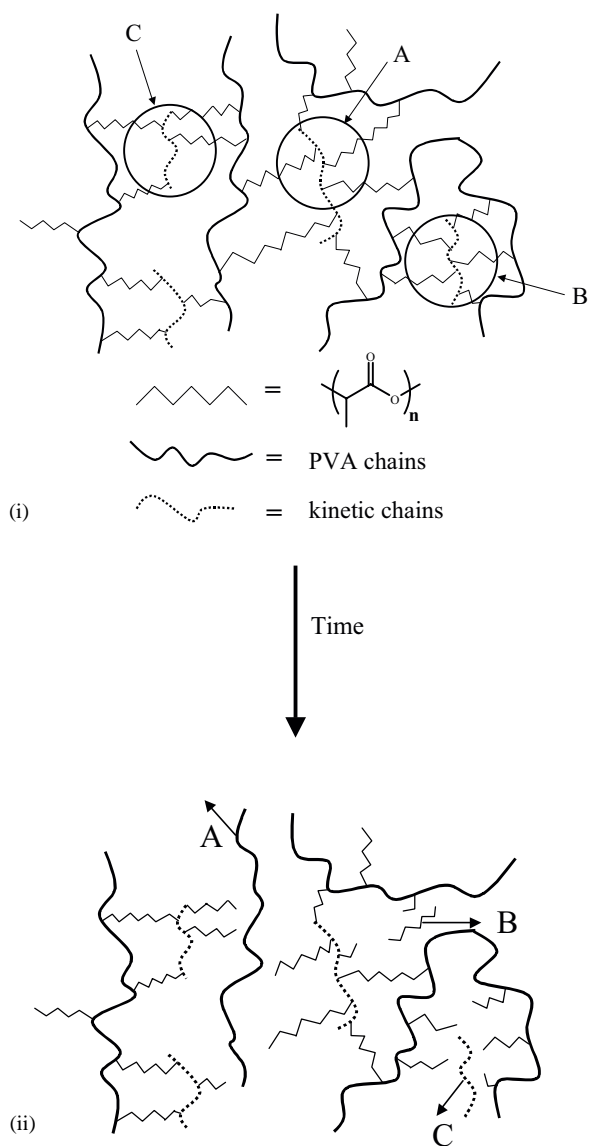


Fig. 3. (i) Network formed upon polymerization of PVA-Deg showing possible crosslinking configurations: (A) reactive groups from three different PVA chains contribute to crosslinking of the network; (B) cyclization caused by reactive groups of the same PVA molecule reacting with one another; and (C) multiple links to the kinetic chains from the same PVA chain. (ii) Possible mass loss mechanisms of crosslinked PVA-Deg. (A) At least one linkage in all crosslinks of a single PVA chain degrade, allowing the entire polymer chain to diffuse out of the network, (B) two or more linkages on the same HEMA-Lac-Suc side chain degrade, allowing release of the entire side chain, and (C) at least one linkage of all side chains connected to the same kinetic chain degrade, releasing the kinetic chain from the network.

sol fraction and is important to characterize for applications that rely on *in situ* gel formation.

3.5. Degradation studies

Fig. 3(ii) depicts several degradation pathways that crosslinked PVA-Deg can undergo. Degradation occurs

via simple hydrolysis of the ester groups of the HEMA-Lac side chains. For mass to be lost by the mechanism shown in Fig. 3(ii, A) (i.e., by the PVA backbone diffusing out of the network), at least one ester bond of all HEMA-Lac crosslinks connected to that PVA chain must be hydrolyzed. If multiple ester bonds of a single crosslink degrade, parts of that crosslink can diffuse out of the network, as shown in Fig. 3(ii, B). Finally, the kinetic chain can diffuse out of the network, as shown in Fig. 3(ii, C), if at least one ester bond from all of the HEMA-Lac side chains connected to the kinetic chain are degraded. A review of the degradation of multi-functional crosslinking molecules has been published by Martens et al. [19].

Depending upon the composition of the starting macromer solution, the crosslinked PVA-Deg scaffolds will primarily lose mass by the three mechanisms outlined in Fig. 3(ii). The probability that a given portion of the network (either HEMA-Lac crosslinks, kinetic chains, or PVA chains) will diffuse out of the network depends on the number of links that are holding that segment into the network. If the kinetic chains are short compared to the functionality of the PVA macromer, it is more probable that the kinetic chains will diffuse out of the network since they are held into the network by fewer degradable linkages. In all networks, only two ester bonds must be broken for a segment of poly(lactic acid) to become unattached from the network. Therefore, the relative proportion of mass loss via mechanism B in Fig. 3(ii) will be directly proportional to the mass fraction of poly(lactic acid) in the original macromer solution. In all cases, one expects that HEMA-Lac chains or fragments would erode from the network the quickest since only two ester bonds must be degraded before a HEMA-Lac fragment can be released.

Fig. 4 demonstrates typical experimental results for the mass swelling ratio (q) of hydrogels formed from these multifunctional PVA macromers as a function of degradation time, and the percent mass loss as a function of time is shown in Fig. 5. These figures illustrate the general trends observed in the macroscopic behavior of these PVA-Deg networks during their degradation. As links in the network slowly hydrolyze, the structure of the network dramatically changes. Initially, the network is highly crosslinked; however, as ester bonds cleave, the network begins to swell, taking up more water. Hydrogels are composed of water-soluble polymers held tightly together, forming an insoluble matrix. When crosslinks in the network are cleaved, the network swells and imbibes more water due to a thermodynamic driving force. Simultaneously, mass is lost due to the mechanisms illustrated in Fig. 3(ii). Assuming pseudo-first-order degradation kinetics of the degradable blocks, the swelling ratio as a function of time can be fit with an exponential function. The data

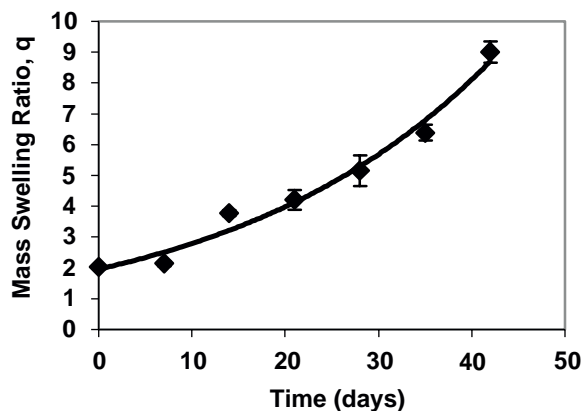


Fig. 4. Experimental mass swelling ratio, q , as a function of degradation time for macromer D (6K PVA, 8 lactic acid units per side chain, 6% substitution, polymerized from a 40% macromer solution in DMSO).

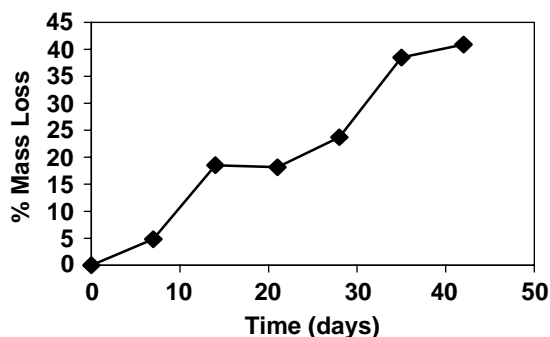


Fig. 5. Percent mass loss as a function of degradation time for macromer D (6K PVA, 8 lactic acid units per side chain, 6% substitution, polymerized from a 40% macromer solution in DMSO).

shown in Fig. 4 show good agreement, and similar exponential increases in the swelling ratio are seen with other degradable hydrogels [20]. Since the mass swelling ratio is the ratio of wet mass to dry mass, the swelling ratio gradually increases with time. Ultimately, reverse gelation occurs. At this point, q goes to infinity as the network reaches a point where it is no longer a continuous network but is composed of highly branched, soluble fragments. At reverse gelation, mass loss is complete as the entire network dissolves.

Fig. 5 shows the corresponding mass loss from this network with degradation time. Typically, there is a rapid initial mass loss (on the order of several hours) that is not captured in these degradation results. This initial mass loss is due to the sol fraction diffusing out of the network and does not represent actual mass loss due to hydrolysis of the network crosslinks. Degradation controlled mass loss occurs when kinetic chains, PLA fragments, and PVA chains are released and diffuse from the network. Depending upon the relative lengths

of the kinetic chains vs. the functionality of the PVA-Deg macromer, the overall mass loss profile can vary significantly. For example, if the kinetic chains are small and held into the network with only a few crosslinks, and the PVA chains are highly functionalized (e.g., have 20–25 side chains per chain) and attached to the network by numerous crosslinks, then the probability that the kinetic chains are released earlier in the degradation is far greater than the probability of releasing the PVA chains. In Fig. 5, the hydrogel was formed from a macromer with ~ 8 double bonds per PVA chain, so the initial mass loss is likely dominated by the release of the PVA, rather than the kinetic chains from the network. For this hydrogel, $\sim 50\%$ of the mass is contained in the PVA chain, while $\sim 45\%$ resides in the degradable PVA units and only $\sim 5\%$ in the kinetic chains. With time, the percent mass loss increases until a reverse gelation point is reached. At this point, the mass loss rapidly approaches 100% (not shown), as the gel becomes a collection of highly branched, but soluble, chains.

The results shown in Figs. 4 and 5 are typical of the degradation behavior observed in highly swollen and degradable hydrogels. However, the rate of swelling (q) and degradation (percent mass loss) can vary greatly among the gels, depending upon such factors as the percent substitution, number of lactic acid repeat units per side chain, and macromer solution concentration during network formation. Fig. 6 illustrates the effect of the number of lactic acid repeat units on the mass loss profile, keeping all other variables (percent substitution, PVA molecular weight, and macromer solution concentration) constant. As seen in Fig. 6, increasing the number of lactic acid repeat units causes a decrease in the rate of mass loss from these gels. Previous results with PEG-based macromers have observed the opposite behavior; increasing the number of lactic acid repeat units in the degradable blocks increases the rate of

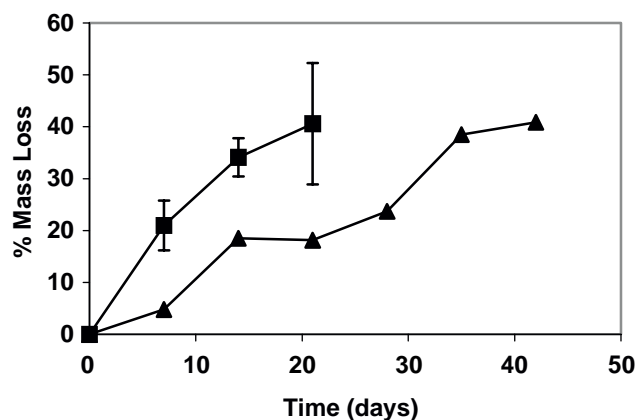


Fig. 6. The percent mass loss as a function of degradation time for networks formed from macromers A (■, 4 lactide repeat units, 5% substitution, 40% macromer solution) and D (▲, 8 lactide repeat units, 6% substitution, 40% macromer solution).

degradation and subsequent mass loss [20]. Since only one of the degradable groups within the PLA block must be hydrolyzed to break a crosslink, increasing the number of lactic acid repeat units should increase the probability of releasing the attached segments.

However, in these PVA gels, increasing the number of lactic acid repeat units significantly alters the hydrophobicity of the network. Lactic acid groups are relatively hydrophobic, and it is likely that these chains interact with one another via London dispersion forces and through other hydrophobic interactions in an aqueous buffer. As a result, the lactic acid chains may exclude water, preventing the hydrolysis of the esters, slowing degradation. For example, macromer A (4 lactide repeat units, 5% substitution, 40% macromer solution) and macromer D (8 lactide repeat units, 6% substitution, 40% macromer solution) have approximately the same percent substitution, but the latter has twice as much lactide content. The initial mass swelling ratios (a measure of hydrophobicity) are 10.0 for macromer A vs. 2.0 for macromer D, indicating that macromer D is much more hydrophobic. A lower equilibrium water content can slow the degradation rate and the overall mass loss, since diffusion of released species from the gel will also decrease. It is likely that the same mechanism occurs to some extent with fewer number of lactic acid repeat units (i.e., four repeat units), but there is simply a decreased ability of those chains to exclude water due to the high hydrophilicity of PVA. This hypothesis would explain the observed behavior in Fig. 6. An alternative explanation for the observed mass loss profiles is that the gel fraction of macromers A and D may be quite different, indicating dissimilar initial network structures and crosslinking densities. Although the sol fraction for A was not measured, the sol fraction of macromer D was the highest of those measured, and its high crosslinking

density, as compared to other macromers, contributes to its slower erosion.

The functionality of the macromer (i.e., the percent substitution of photocrosslinkable, degradable side chains) can also impact the network hydrophobicity, structure, and the resulting mass erosion profile. Fig. 7 plots the percent mass loss as a function of degradation time for gels formed from two macromers with increasing functionality (the percent substitution was varied from 3% to 6%). As can be seen in Fig. 7, the percent substitution increases the initial crosslinking density of the network and decreases the rate of mass loss. Since the PVA chains in gels formed from macromer E are connected to the network by fewer crosslinks than those from macromer D, the mass loss from the network is quicker. Thus, percent substitution on the PVA macromer is one parameter that can be utilized to significantly alter not only the initial crosslinking density and macroscopic properties, but also the mass erosion profile of the PVA-Deg scaffold.

Table 3 summarizes the total degradation time for hydrogels synthesized from each of the macromers and polymerized under a range of conditions. Specifically, the number of lactide repeat units per crosslinkable side chain, percent substitution, and percent macromer in solution were examined. Macromer F degraded the fastest (less than a week) due to the low macromer solution concentration of 20% and percent substitution, which combine to form a loosely crosslinked gel. Networks composed of this macromer contain fewer total numbers of crosslinks that must be degraded as compared to other macromers that were polymerized at a higher concentration. Macromer D (8 lactide repeat units, 6% substitution, 40% macromer solution) did not degrade completely until about 11 weeks. The slow degradation of these networks, as compared to those with lower HEMA-Lac content (macromers E and F), can be attributed to the higher crosslinking density of the gels as well as the large fraction of hydrophobic HEMA-Lac (0.67) (in relation to the PVA component (0.33)), which decreases the water content of the gel.

The last column of Table 3 gives the initial mass swelling ratios of the macromers studied. The initial mass swelling ratio is influenced by both the network crosslinking density and the hydrophobicity of the PLA grafts. Experimental results show that the macromers with increased hydrophobicity exhibit smaller initial q values than more hydrophilic macromers. The most hydrophobic macromer studied, D, has the smallest initial q (2.0), while macromer A, the most hydrophilic studied, has the largest initial q (10.0). There is a distinct decrease in initial mass swelling between macromers A and B (10.0 and 2.75), which is likely due to the increased substitution on macromer B. Based solely on hydrophobicity, one would expect macromer C to have a lower initial q than macromer B. However, macromer

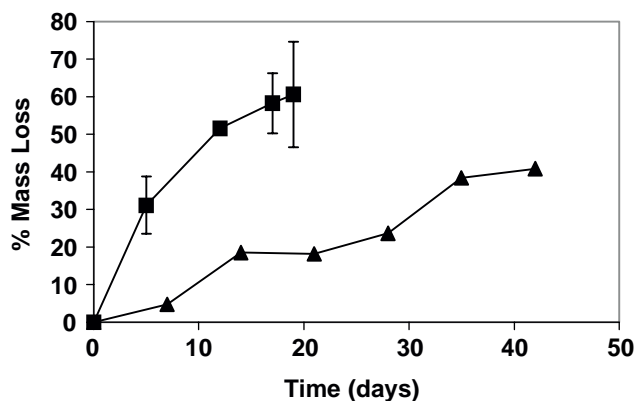


Fig. 7. The percent mass loss as a function of degradation time for networks from macromers D (▲, 8 lactide repeat units, 6% substitution, 40% macromer solution) and E (■, 8 lactide repeat units, 3% substitution, 40% macromer solution).

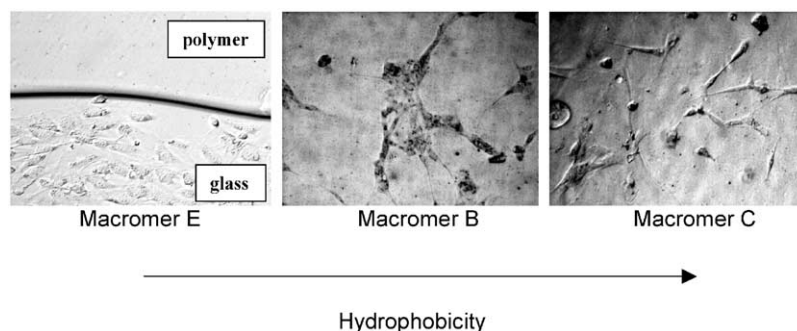


Fig. 8. Valve interstitial cells seeded on macromers E (8 lactide repeat units, 3% substitution, 40% macromer solution) (left, 10 × magnification), B (4 lactide repeat units, 7.5% substitution, 40% macromer solution) (middle, 20 ×), and C (4 lactide repeat units, 17% substitution, 15% macromer solution) (right, 20 ×). Cell attachment improves from left to right, corresponding to an increase in hydrophobicity.

C was polymerized from a more dilute solution (15%), yielding lower crosslinking density, and greater mass swelling.

3.6. Cell attachment to PVA-Deg

Fig. 8 shows cells attached to gels of increasing hydrophobicity. In general, greater hydrophobicity corresponds to better valve interstitial cell (VIC) attachment. In the left panel, no cells attached to the polymer (glass was used as a control surface). In the center panel, cells are attached, but are clumped together, and in the right panel, most cells are attached and have assumed the long, spindle morphology characteristic of valve cells. However, they proliferated at a slower rate than VICs grown on tissue culture polystyrene, which is typical for most degradable substrates. It is believed that the improvement of VIC adhesion seen in Fig. 8 is due to greater adsorption of cell adhesion proteins on the more hydrophobic PVA gel compositions.

4. Conclusions

A second generation of PVA-based scaffolds was developed that integrates the advantages of PVA (high water content, tissue-like elasticity, and ability to attach a variety of molecules) with those of PLA (degradability, ability to photocrosslink, and hydrophobicity). The number of lactide repeat units per side chain, percent substitution, and macromer concentration all affect the rate of mass loss of these degradable PVA gels. This material has great potential as a tissue engineering scaffold due to the ability to control the rate of network degradation, mass erosion profile, and its bulk chemical properties (i.e., hydrophobicity). Cell attachment was found to improve by incorporating a greater fraction of hydrophobic HEMA-Lac chains into the gels. Specifically, valve interstitial cells were seeded on two-

dimensional surfaces of various compositions of PVA-Deg. The increased cell attachment was attributed to the increased adsorption of cell adhesion proteins to hydrophobic gels, thereby promoting the adherence of cells to the polymer surface.

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