Oligo-α-hydroxy Ester Cross-Linkers: Impact of Cross-Linker Structure on Biodegradable Hydrogel Networks

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ABSTRACT: We describe the synthesis of a series of biodegradable oligo-α-hydroxy ester cross-linkers and evaluate their impact on the degradation kinetics and macromolecule diffusion from a hydrogel network. By changing the steric and electronic environment at the site of degradation in the cross-linker, we were able to modulate the degradation, swelling kinetics, and corresponding release profiles of macromolecules from poly(HPMA) hydrogel networks under physiologically relevant conditions. As the steric hindrance and electron demand at the site of hydrolysis for three different cross-linkers was increased, the total time for the hydrogel network to completely dissolve increased from 2 to over 30 days while incubated in pH 7 buffer. As the number of hydrolyzable sites and the electron demand at the side of hydrolysis decreased, the time to completely dissolve decreased from weeks to several days. Increasing the cross-linking density for one of the degradable cross-linkers (1.5% to 3.0% feed ratio) increased the degradation time by several weeks. Burst release was absent for high molecular weight solutes because the release rate depended on controlled degradation of the polymer network and an increase in average network mesh size. The synthetically adaptable cross-linkers described herein offer a new approach for controlling the rate and extent of release from biodegradable hydrogel networks.

Introduction

Biodegradable hydrogels offer a promising approach for the controlled release delivery of small molecules and macromolecules.[1,2] They offer a safe and effective means for controlling the release kinetics of entrained agents and the biodistribution of a drug.[3−5] The cross-linker plays an important role in determining the swelling and pore size of the hydrogel matrix, making it an ideal target for synthetic modifications to control the corresponding release kinetics of entrained molecules.[6−8]

A significant amount of work has been directed toward developing cross-linker technology for biomedical applications.[2,9] Previous research has demonstrated that the mechanism of solute release from degradable cross-linked polymers is impacted by a variety of factors, including pH, solute molecular weight, cross-link density, enzymes, and hydrolytically labile spacers.[1,7,8,10,11] Water-soluble biodegradable macromolecular cross-linkers have been the subject of study by several groups. These polymers are designed to contain α-hydroxy ester monomers, such as lactate and glycolate esters for degradability,[5] and are attached to poly(ethylene glycol) (PEG)[12] or dextran.[6,7,13−17] They are particularly well suited for biological applications because they are readily synthesized and degrade under physiological conditions into biocompatible products. More recently, macromolecular cross-linkers have been synthesized which contain short oligomeric sections of α-hydroxy esters terminated in vinyl groups.[13] Similarly, small molecule cross-linkers composed of acid labile groups,[18] anhydrides,[19,20] and peptides[21] have been studied for anticancer, dental, and orthopaedic applications.

In this work, we describe a new class of synthetic cross-linkers composed of symmetrical oligo-glycolate and oligo-lactate esters terminated with vinylic 2-hydroxypropyl methacrylamide (HPMA) polymerizable moieties[22] (Figure 1). The incorporation of these cross-linkers, which have homogeneous degradation kinetics, into a hydrogel network permits control over the degradation and release profile of the bulk polymer under biologically relevant conditions (Figure 1a−c). We selected the carboxylic ester functionality to be the cleavable moiety because this ester has a well-understood mechanism of hydrolysis,[23] and its kinetics can be controlled under physiological conditions by varying the electronic and steric factors at the site(s) of hydrolysis. Here we report a series of homologous cross-linkers that is readily synthesized and purified, is comprised of biocompatible components, and can be easily adjusted to degrade with a range of time constants under physiological conditions. We then designed these cross-linkers (Figure 1d) based on the hypothesis that by varying the steric environment of the cleavable ester moiety and the number of such moieties (Table 1) within a cross-linker, as well as the cross-link density within the network, we could create hydrogel systems with a wide range of degradation and release rates.[2,11,13,24] Advantages of these small molecule cross-linkers in comparison to polydispersed macromolecular cross-linkers are their ease of synthesis and characterization

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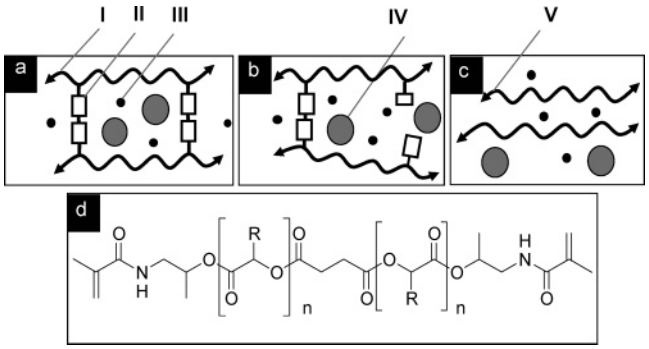

Figure 1. (a) Schematic diagram showing a cross-linked element of a degradable hydrogel: (I) linear polymer backbone p(HPMA); (II) the degradable region of the cross-linkers; (III) water which swells the gel and aids in degradation; (IV) entrained macromolecule. (b) Degradation of the polymer and an increase in the mesh size of the polymer. (c) Final product of linear polymer (V) and release of macromolecule. (d) General structure of the cross-linkers in which n and R refer to the number of α-hydroxy esters on each side of the diacid and substitution at the α-hydroxy ester, respectively.

Table 1. Description of the Cross-Linkers

compd ID	n	R	name	degradation rate
1	0	NR	$HPMA_2$succinate	ND, control
2a	1	CH_3	$(HPMALac)_2$succinate	slowest
2b	1	H	$(HPMAGly)_2$succinate	medium
3	2	H	$(HPMAGlyGly)_2$succinate	fastest

which could be of benefit in fundamental studies of degradable polymer network behavior and in regulatory considerations related to devices built with these substances.

Experimental Section

Materials and Instrumentation. All chemicals were reagent grade and were used without purification unless otherwise noted. Dichloromethane was distilled from P_2O_5 and stored over 4 Å molecular sieves. All other solvents were obtained in their anhydrous state or stored over 4 Å molecular sieves before use. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100.4 MHz, respectively, on a Varian INOVA-400 spectrometer. Solvent mixtures are given in volume-to-volume ratios unless otherwise stated. Flash chromatography was performed on SiO_2 Kieselgel 60 (70−230 mesh, E. Merck). The FITC dextrans used in release studies were obtained from Sigma-Aldrich.

Cross-Linker Synthesis. 2 equiv of benzyl lactate (or benzyl glycolate) is reacted with succinyl chloride in the presence of pyridine, providing a diester (see Figures 1d and 2). Reductive deprotection of the carboxylate groups of the diester renders a dicarboxylic acid. Addition of HPMA to the dicarboxylic acid activated via carbonyl diimidazole (CDI) provides a diacrylate $(HPMALac)_2$succinate, **2a**, and $(HPMAGly)_2$-succinate, **2b**. The length of the cross-linker can be extended by reacting the diacid intermediate with an additional 2 equiv of benzyl glycolate (or benzyl lactate) and repeating the deprotection step and lastly coupling with HPMA, forming for example $(HPMAGlyGly)_2$succinate, **3**. Cross-linker **1**, which is lacking lactate or glycolate ester moieties, was prepared as a control compound since its hydrolysis rate at pH 7 was expected to be negligible.[25] A sample preparation for the degradable cross-linker **2a** in Figure 1 is given below.

Preparation of $(HPMALac)_2$Succinate, 2a. Compound **5a** (2.2 g, 8.3 mmol) was dissolved in dichloromethane (30 mL) and cooled to 0 °C under an argon atmosphere in a three-necked flask equipped with a stir bar and a powder addition funnel. The reaction vessel was then charged with carbonyl diimidazole (CDI, 2.75 g, 17.0 mmol) via the powder addition funnel (warning: CO_2 gas is released). The reaction vessel was allowed to warm to 25 °C, and then HPMA (2.57 g, 17.0 mmol) was added. The reaction was stirred at 25 °C for 2 h and then washed with 1 M NaH_2PO_4 (10 mL), saturated Na_2CO_3, (10 mL), and brine (10 mL). The dichloromethane phase was then dried (Na_2SO_4) and concentrated in vacuo to give a pale yellow, viscous oil. Yield of **2a**: 4.08 g (95%). Although the purity was >90% by TLC and NMR, the purity could be improved by flash chromatography. Elution on silica gel (300 mL) using 3% methanol/dichloromethane resulted in 3.22 g (75%) of **2a**: $[\alpha]_D = -21.3$ ($c = 1.0$, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.24−1.29 (m, 6H), 1.47−1.51 (m, 6H), 1.96 (s, 6H), 2.70−2.74 (m, 4H), 3.20−3.38 (m, 2H), 3.57−3.72 (m, 2H), 4.87−5.00 (m, 2H), 5.03−5.16 (m, 2H), 5.33−5.36 (m, 2H), 5.71−5.75 (m, 2H), 6.25−6.55 (m, 2H). ^{13}C NMR (62.9 MHz, DMSO-d_6, several peaks exhibited duality which may be due to diastereomers): δ 16.53, 17.21, 17.37, 18.55, 28.20, 42.99, 54.88, 68.74, 70.17, 70.22, 119.11, 139.83, 139.87, 167.68, 167.83, 169.72, 169.89, 171.27, 171.35. HRMS (FAB+) Calcd for $C_{24}H_{27}N_2O_{10}$ (M + H) 513.2448. Found: 513.2418. The complete synthetic methodology for each cross-linker will be published elsewhere.

Gel Synthesis and Testing. The vinyl groups on the terminus of the cross-linking monomer can be used to form a gel network structure. Gels were synthesized using the ammonium persulfate (APS) *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMED) couple as the initiation system.[6,11] The gels described below were made at a mole feed ratio of 1.5 mol % cross-linker as a copolymer with 98.5 mol % HPMA. Before the gels were polymerized, three 1.0 mL plastic syringes, to be used as a slab gel template, were silylanized by briefly incubating them in a heptane solution containing Sigmacote and oven-drying at 90 °C. A representative procedure to form gels was as follows: a 7 mL test tube was charged with HPMA (2.115 g, 14.8 mmol, $[HPMA]_{final} = 5$ M), and the cross-linker was placed on the end of a tarred spatula (109.0 mg, 0.225 mmol, $[XL]_{final} = 0.075$ M). The end of the spatula was submerged in the HPMA, and DI water (1.5 mL) was added to the mixture and then was sonicated until all of the material was dissolved. To this solution was added a solution of APS in water (99 mg, 0.438 mmol, 166 μL of a 2.63 M solution, $[APS]_{final} = 0.143$ M). To this mixture was then added TMED to initiate the polymerization (49 mg, 0.429 mmol, 204 μL of a 2.10 M solution of TMED adjusted to pH 7 with HCl). Immediately after the TMED was added, the mixture of monomers and APS was vigorously mixed on a vortexer for 15 s and then drawn into the 1.0 mL plastic syringes (which act as a mold for gel formation) by plunger aspiration. The gels were allowed to polymerize for 4 h. The gels were cut into 10 mm tall cylinders and were placed in 15 mL vials containing the desired buffer (10 mL). The initial dry mass of the gel was determined by drying seven of the gels from each composition in their relaxed state. The incubation solutions were changed each time the gel was weighed. The gels were incubated in a gyratory water-bath shaker. The temperature was regulated to be 37 ± 2 °C, and the shaker was set to 30 rpm. The change in volume of the polymer network was measured by weighing the gel at different time points. Knowing the initial mass of the dry polymer and the final swollen mass, the swelling ratio Q_v can be determined by eq 1[10]

$$Q_v = \nu_{2,s}^{-1} = \frac{V_s}{V_2} = \frac{V_2 + V_1}{V_2} = 1 + \frac{(m_t - m_2)\rho_1^{-1}}{m_2\rho_2^{-1}} \quad (1)$$

where $\nu_{2,s}^{-1}$ is the polymer volume fraction in the swollen state, V_s is the total volume of the gel in the swollen state, V_2 is the initial volume of the dry gel network, V_1 is the volume of water entrained in the gel, m_t is the total mass of the gel, ρ_1 is the density of water, m_2 is the initial dry mass of polymer, and ρ_2 is the density of the polymer.[26,27] Therefore, by measuring the mass of the swelling gel during its degradation, the swelling kinetics were obtained.

Dextran Solution Preparation, Calibration, and Release Measurements. Three separate stock solutions containing FITC-labeled dextrans of 12, 42, and 148 kDa were prepared at concentrations of 28.5, 20.9, and 24.1 mg mL^{-1},

respectively. These solutions were then used as above to construct the monomer solutions for polymerization. The release of FITC dextran was measured by UV/vis spectrophotometry (Perkin-Elmer Lambda 2S UV/vis spectrophotometer). Samples of ~1 mL of solution were drawn into cuvettes, and the absorption spectrum for each sample was measured over a 350−600 nm range. The intensity value was recorded at the absorption maximum, and each measurement was rescaled to a constant baseline, since there was variation in the initial baseline of many of the spectra. The measured value of the absorption at any one time was then normalized according to the maximum amount released. In the case of degradable hydrogels that exhibited complete degradation behavior, the entire quantity of fluorescent dextran was released into solution, or equivalently 100% of the contents was measured at the final measurements when degradation occurred. For the control hydrogels that did not undergo degradation, 100% release was mathematically determined on the basis of the expected calculation of 100% release from the initial unpolymerized solution.

Estimation of Network Mesh Size. Mesh size has been estimated on the basis of equilibrium swelling for hydrogels composed of PVA, PEG, and PHEMA backbones.[14−16,24] The procedure for calculating mesh size, adopted from Canal and Peppas[24] (Figure 5b), is as follows. We used eq 2 to relate the polymer swelling to the molecular weight between cross-links, $\bar{M}_c$, in the network,[28,29] thereby linking the volume fractions of polymer before and after swelling, $v_{2,r}$ and $v_{2,s}$, to the cross-link density. However, this equation was only used with gels made of cross-linkers **2a** and **2b** which degraded slowly enough to be in equilibrium.

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{\bar{v}/\bar{V}_1[\ln(1 - v_{2,s}) + v_{2,s} + \chi {v_{2,s}}^2]\left[1 - \frac{1}{N}({v_{2,s}}^{2/3})\right]^3}{v_{2,r}\left[(v_{2,s}/v_{2,r})^{1/3} - \frac{1}{2}(v_{2,s}/v_{2,s})\right]\left[1 + \frac{1}{N}(v_{2,s}/v_{2,s})^{1/3}\right]^2} \quad (2)$$

In this equation, $\bar{M}_n$ is the number-average molecular weight of the polymer in the polymer network which was determined experimentally to be $\bar{M}_n = 93\,000$ for these pHPMA networks by degrading the hydrogel samples at pH 12.0, dialyzing the solution in a 500 MW cutoff membrane, and measuring the molecular weight distribution by GPC with a light scattering detector (Wyatt Technologies). The value of $\bar{v}$ is the specific volume of HPMA (0.841 cm^3/g), $\bar{V}_1$ is the molar volume of water (18.14 cm^3/mol), and χ is the Flory−Huggins parameter. $v_{2,r}$ was experimentally determined to be 0.57 as an average for gel compositions studied. The value of χ for poly(HPMA), given by eq 3, appears in the literature[27] and is an empirical function of the polymer volume fraction $v_{2,s}$ and the temperature. It is given by the relation at 20 °C[27]

$$\chi = 0.473 + 0.489 v_{2,s} \quad (3)$$

N represents the number of bond vectors in the chain and is given by eq 4, where M_r is the molecular weight of the monomer unit (144 g/mol).

$$N = \frac{2\bar{M}_c}{M_r} \quad (4)$$

This parameter is added to account for the random non-Gaussian distribution of the effective chains.[30,31] To calculate the mesh size, ξ, the number-average molecular weight between cross-links, $\bar{M}_c$,was used to estimate the end-to-end distance of the unperturbed (or solvent-free) state $(\bar{r}_0^2)^{1/2}$ along with the characteristic ratio, C_n, of the HPMA chain, as shown in eq 5.

$$({\bar{r}_0}^2) = l(2\bar{M}_c/M_r)1/2{C_n}^{1/2} \quad (5)$$

In this equation the C−C bond length is given by l (1.54 Å), and the characteristic ratio C_n was chosen to be 10. We were unable to find the characteristic ratio of HPMA in the literature, and therefore we choose an average value of 10 because HPMA is expected to have a characteristic ratio between HEMA ($C_n = 6.9$), a relatively hydrophobic polymer, and PEG ($C_n = 14$),[32] a more hydrophilic polymer. Mesh size, ξ, was then computed as shown in eq 6. The Stokes radii for the fluorescent FITC dextrans used in our studies were obtained from the Sigma-Aldrich catalog.

$$\xi = {\nu_{2,s}}^{1/3}({\bar{r}_0}^2)^{1/2} \quad (6)$$

Results and Discussion

Three new degradable symmetrical cross-linkers containing oligo-glycolate and oligo-lactate esters (Figures 1d and 2) were synthesized according to the scheme depicted in Figure 2 and copolymerized with 2-hydroxypropyl methacrylamide (HPMA), using free radical polymerization to give biodegradable hydrogels (Figure 3). The swelling kinetics (measured swelling ratio Q vs time) of the four gel systems were measured at intervals in pH 7.4 media (Figure 3). Figure 3a shows a photograph of biodegradable gels composed of 1.5 mol % cross-linker **2b** $(HPMAGly)_2$succinate and 98.5 mol % HPMA, after incubation at pH 7.4 for 2, 5, and 15 days. These experiments demonstrate the well-known effect that cross-link density has on the degree of equilibrium gel swelling.[10] As the cross-linker was hydrolyzed, the pore size increased and the elastic modulus decreased, allowing increasing amounts of water to be imbibed by the hydrogel. Ultimately, the gels dissolved into water-soluble components, completing a gel to sol transition. As can be seen in Figures 3a and 4, the structure of the cross-linker has a large impact on the rate of this process.

Figure 4 graphically illustrates the range of rates of degradation that can be obtained from the cross-linkers. In Figure 4, it can be seen that cross-linker **1** behaves in a static fashion similar to known nondegradable cross-linkers. As described above, the degradation rate, which is set by the chemical structure of the cross-linker, determines the swelling ratio. Finally, by simply manipulating the initial concentration or mole fraction of cross-linker in the network, one can also adjust the time course to allow for faster or slower overall gel degradation rates because there is more cross-linker in the network to degrade.

The slowest gel of the series dissolved after ~800 h (~30 days) and was composed of cross-linker **2a**, which had a single lactate ester on each side of the succinate core. In comparison, the unmethylated homologue of this material, cross-linker **2b**, dissolved after ~350 h (~14.6 days), which indicated that this cross-linker was cleaved at nearly twice the rate as cross-linker **2a**. We attribute this to differences in the steric crowding at the site of reaction during attack by the hydroxide nucleophile.[33] The methyl group in **2a** increases crowding, resulting in slower ester hydrolysis than **2b**. Not surprisingly, the fastest cross-linker to degrade was 3, which swelled and dissolved after 50 h (~2.1 days). In comparison to cross-linker **2b**, this gel was the more labile due to increased electronic demands on the carboxylate groups resulting from the additional glycolate functionality and the statistical effect of a greater number of labile sites. These factors in concert significantly increased the overall rate of degradation. Finally, by simply manipulating the initial concentration or mole fraction of cross-linker in the network, one can also

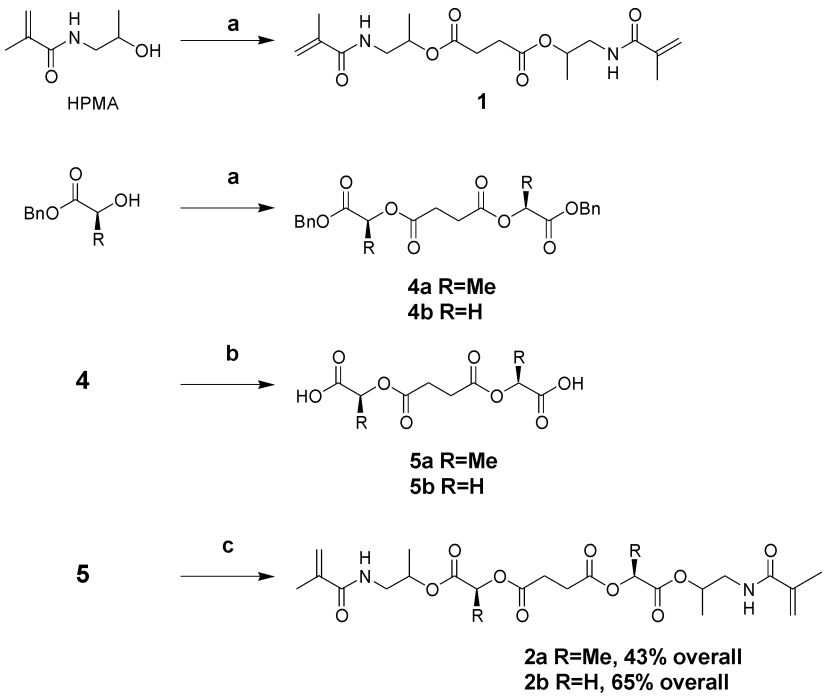

Figure 2. Conditions for the synthesis of the cross-linkers. Conditions: (a) succinyl dichloride, CH_2Cl_2, pyridine, 0 °C; (b) Pd/C, 50 psi of H_2, *i*-PrOH; (c) DMF, CDI, 0 °C, HPMA.

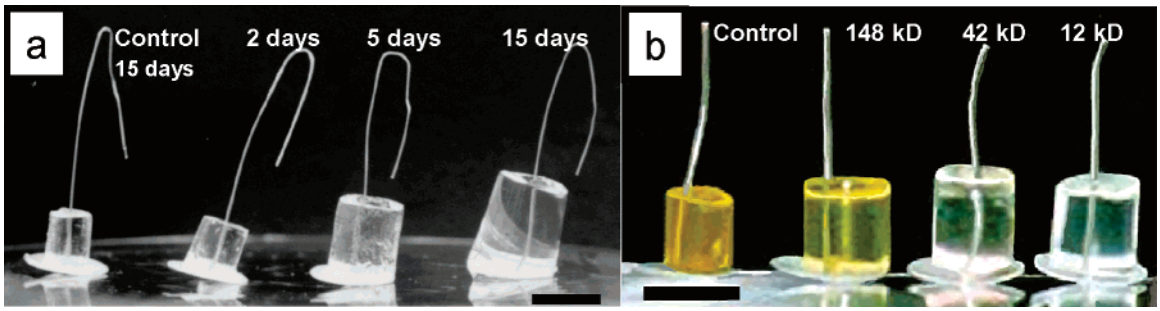

Figure 3. (a) Photograph of the swelling profile for biodegradable hydrogels composed of cross-linkers **2b**,[8,22] $(HPMAGly)_2$succinate (X = 1.5 mol % cross-linker feed ratio and 98.5% HPMA), after incubation in pH 7.4 buffer (100 mM phosphate buffer, ionic strength of 200 mM; T = 37 °C). From left to right: control gel made with nondegradable cross-linker **1** ($HPMA_2$succinate) after 15 days and gel made with cross-linker **2b** after 2, 5, and 15 days, respectively (see Figure 4 for swelling profiles of hydrogels composed of cross-linkers **1**−**3**). (b) Photograph displaying the dynamic release of entrained macromolecules: four hydrogel samples (3.0 mol % feed cross-linker and 97 mol % HPMA) incubated at pH 7.4 solution for ~300 h (13 days) containing three different molecular weights of dextran. From left to right: nondegradable control (cross-linker **1**) containing 148 kDa fluorescenated dextran and cross-linker **2b** with 148, 42, and 12 kDa fluorescenated dextran samples, respectively. Scale bars: 1 cm (see Figure 5a for release profiles).

adjust the time course to allow for faster or slower overall gel degradation rates. As shown in Figure 4, by doubling the amount of cross-linker **2b**, we were able to dramatically decrease the rate of change of the swelling ratio and thus obtain a total degradation time nearly equal to the slowest cross-linker **2a**.

To evaluate the influence of swelling kinetics on the release rate of macromolecules, we synthesized degradable hydrogels with entrained dextrans of varying sizes. This allowed us to study the relationships of a cross-linker's chemical structure, the hydrodynamic size of an entrapped macromolecule, the hydrogel cross-link density, and the pH dependence of degradation on the swelling and release characteristics. Figure 3b shows a photograph of biodegradable gels that were loaded with three different molecular weight fluorescenated dextrans. As the gel dynamically swelled and the cross-linkers degraded, the mesh size expanded, allowing for the release of the entrained macromolecules.

Figure 5a demonstrates that one can control the rate of release from the gel by changing either the cross-linker or the MW of the entrained macromolecule. Of particular interest in Figure 5a is the demonstrated ability to closely match the release profile of a 148 kDa entrained molecule to that of a 42 kDa entrained molecule by simply changing the cross-link density (i.e., 1.5% for the 42 kDa dextran and 3% for the 148 kDa dextran). In this way, it was possible to construct two different gels that released ~50% of their fluorescenated dextran (42 and 148 kDa) at 200 h. Furthermore, by comparing the two cross-link densities (1.5% and 3%) used with the 148 kDa dextran, one can see that after 200 h the 3% cross-linked hydrogel has released only 20% of the macromolecule vs 50% for the 1.5% cross-linked polymer. In the higher cross-link density case, the release profile approximates zero-order release, which is advantageous in many therapeutic dose regimens.[34]

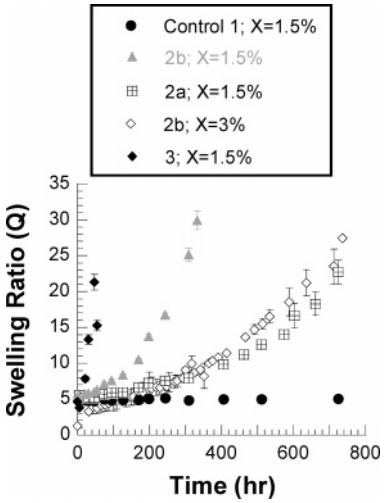

Figure 4. Time course of swelling ratio (Q) of degradable gels made from four different cross-linkers (**1**−**3**) at pH 7.0, 100 mM PBS; I = 200 mM; T = 37 °C, error bars ± SD, n = 6 (X = mol % feed cross-linker). See images in Figure 3a for images of gels made with cross-linker **2b**.

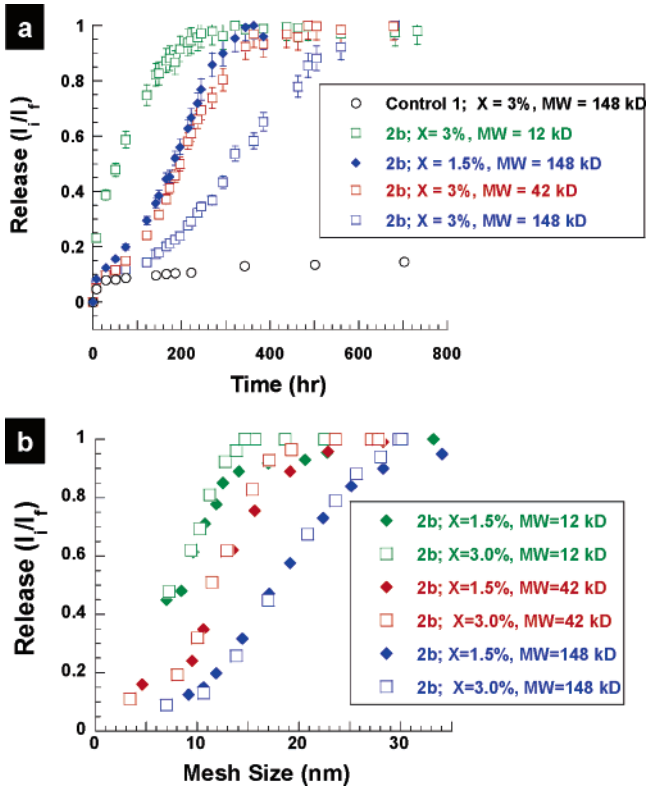

Figure 5. (a) Release rate of FITC dextran from gels composed of 3% (open symbols) and 1.5% (closed symbols) mole feed ratio cross-linker **2b** and p(HPMA) at pH 7.0 (see Figure 3). The control cross-linker was nondegradable. Error bars SD, n = 3. (b) Plot of fractional release as a function of mesh size for a single cross-linker **2b** at pH 7.0 and for various MW FITC dextrans (X = mol % feed cross-linker).

To better understand the molecular basis for the release rates of the different MW dextrans, we estimated the mesh size of the hydrogels at different swelling ratios.[14−16,24] Figure 5b shows the relationship between the release rates and the estimated average mesh size for different MW dextrans and cross-link densities. Consistent with our explanation of the degradation mechanism of the gel, the release behavior for a given mesh size is independent of the gel's initial cross-link density. As a result, fluorescenated dextran molecules of 12, 42, and 148 kDa entrained in six distinct gels are observed to converge into three mesh size/release profiles that depend on the size of the entrained dextran.

Figure 5b also demonstrates how a larger mesh size is required to achieve the release of larger entrained dextran molecules, since 50% release of the 12kD entrained molecules was achieved after the mesh size expanded to 7 nm compared with a 15 nm mesh size required for release of the 148 kDa entrained molecules. Given that the Stokes radius of the 148 kDa entrained dextran molecule is ∼9 nm, Figure 5b illustrates how the 148 kDa dextran is largely contained in the gel until the mesh size reaches 9 nm, at which point release increases significantly. This relationship between mesh size, entrained macromolecule size, and release has clear implications for the design of biodegradable gels engineered for different sized macromolecules in systems that largely avoid burst release.

The results above show that these ester-containing cross-linkers conformed to the known order of importance of steric, electronic, and pH effects in ester hydrolysis.[23] The data confirm that the primary factors that impact gel degradation rates are the steric hindrance and electronic properties at the site(s) of degradation, the number of sites available for hydrolysis, and the degree of initial cross-linking within the network. In comparison to the normal bulk degradation kinetics of PLGA polymers, where the hydrolysis mechanism is dominated by autocatalytic hydrolysis, the degradation mechanism for these polymers is dominated by normal base hydrolysis at pH 7. It is well-known that for the polymers of lactic and glycolic acids base hydrolysis is slower than the corresponding acid hydrolysis. However, this is not true for polymer systems made of these cross-linkers.

Conclusion

We have successfully developed[22] and tested a new class of small molecule biodegradable cross-linkers that offer several advantages over existing similar cross-linking systems.[12,13] First, the methodology allows for the cross-linker structure to be readily modified and easily purified into a single compound after synthesis. Accordingly, the method allows for control over the hydrolysis rate by increasing or decreasing the number of monomers in the oligomer. Third, the end groups of the oligomers can be readily modified to accommodate either condensation or free radical polymerizations. Finally, by varying the cross-linker structure(s), their proportions, and the cross-link density, one can readily "dial in" the degradation and release properties of a hydrogel across a range of several orders of magnitude in time. This opens up a range of material properties that one could imagine using to obtain different controlled release profiles.[7,9] However, loading of therapeutic proteins into gel networks by polymerization in the presence of the protein presents a number of unsolved challenges related to purification and protein stability. We circumvented some of these issues by loading the protein after forming the polymer network using electrostatic interactions between the polymer network and the protein.[35] Biodegradable hydrogels prepared using these synthetically adaptable cross-linkers offer biomaterials researchers a new tool for applications spanning drug delivery, tissue engineering,

and medical devices. Studies examining therapeutic applications and physical behavior of these materials are underway.

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