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Symmetrical biodegradable crosslinkers for use in polymeric devices

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Abstract—There is a need for biodegradable hydrogels that deteriorate at defined rates under physiological conditions for use in engineered tissue constructs and drug delivery. These hydrogels should contain components that are readily synthesized, biocompatible and easily incorporated into hydrogel networks. This need was addressed through a judiciously designed series of crosslinkers composed of symmetrical oligo-glycolate and oligo-lactate esters terminated with vinylic moieties (**1**). These materials were incorporated into poly(HPMA) networks via free-radical polymerization. This work describes the preparation of symmetrical, lactate and glycolate ester based crosslinking agents and their incorporation into a hydrogel network composed of 2-hydroxypropyl methacrylamide (HPMA). By varying the number of lactic and glycolic acid residues ($n = 0, 1, 2$) within the crosslinker, the rate of hydrolytic degradation of the gel can be controlled.

There is an increasing need for biodegradable materials that can be adjusted to degrade over a wide range of rates.[1,2] However, most of the reports on biodegradable materials have focused on water-insoluble hydroxyacid polyesters.[2] Much less work has addressed water-based biodegradable hydrogel systems in which the degradation rate can be tuned and adjusted to the requirements of the application.[3] It has been possible to engineer hydrogel networks that swell in a predictable manner in a given environment (e.g., aqueous solution with variable ionic strength, pH, or temperature). Such materials have been described as environmentally sensitive or 'smart' materials.[4] Being able to control and predict the behaviour of these materials is important for realizing safe and efficient time release of drugs and the development of cell-based tissue scaffolds.[3] In degradable and non-degradable hydrogels, the polymer network is formed by two structural components: crosslinks and polymer chains, hence biodegradability must be built into one or both components. This can be accomplished through the use of two separate structural motifs by placing degradable bonds at either (a) in the crosslinker or (b) in the polymer backbone (Fig. 1).

The mechanisms of degradation of hydrogels can be classified into three basic categories: solubilization,[5] enzyme catalysis,[6] and/or chemical hydrolysis.[3] In the latter case, this has been accomplished by incorporation of chemically labile moieties into the polymeric backbone, using functional groups such as esters,[7] lactones, orthoesters,[8] carbonates, phosphazines,[9] and anhydrides. Alternatively, hydrogels composed of bis-functional, macromonomer crosslinking agents have been described.[2,10]

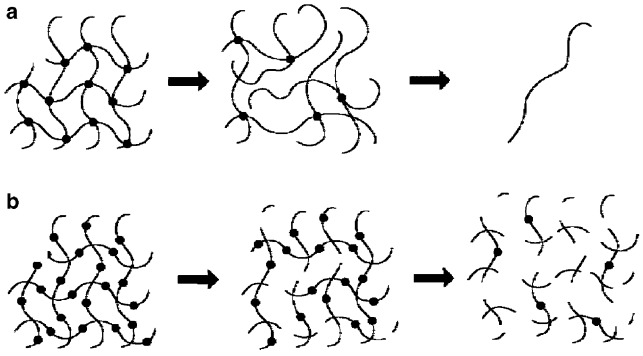

Figure 1. Schematic representation of two different architectural motifs for degradable polymer networks. (a) Degradation of a polymer network resulting from degradable crosslinks. (b) The results from degradable linkages in the polymer backbone.

We sought low molecular weight, biodegradable crosslinkers for incorporation into hydrogel networks to be

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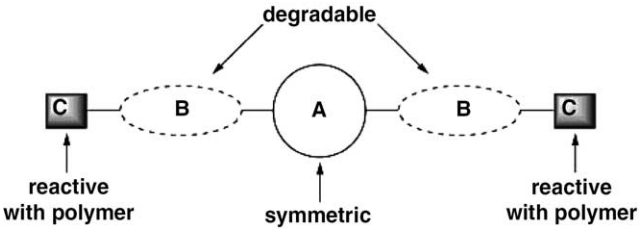
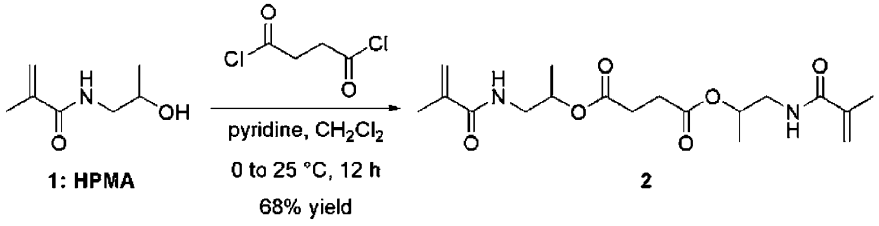


Figure 2. Generalized structure for symmetrical, degradable polymer crosslinking agents. The crosslinker contains a symmetric centerpiece **A** (e.g., succinic acid), biodegradable regions **B** (e.g., lactic or glycolic acids), and reactive terminal units **C** (e.g., ±2-hydroxypropyl methacrylamide: HPMA) for polymer crosslinking.

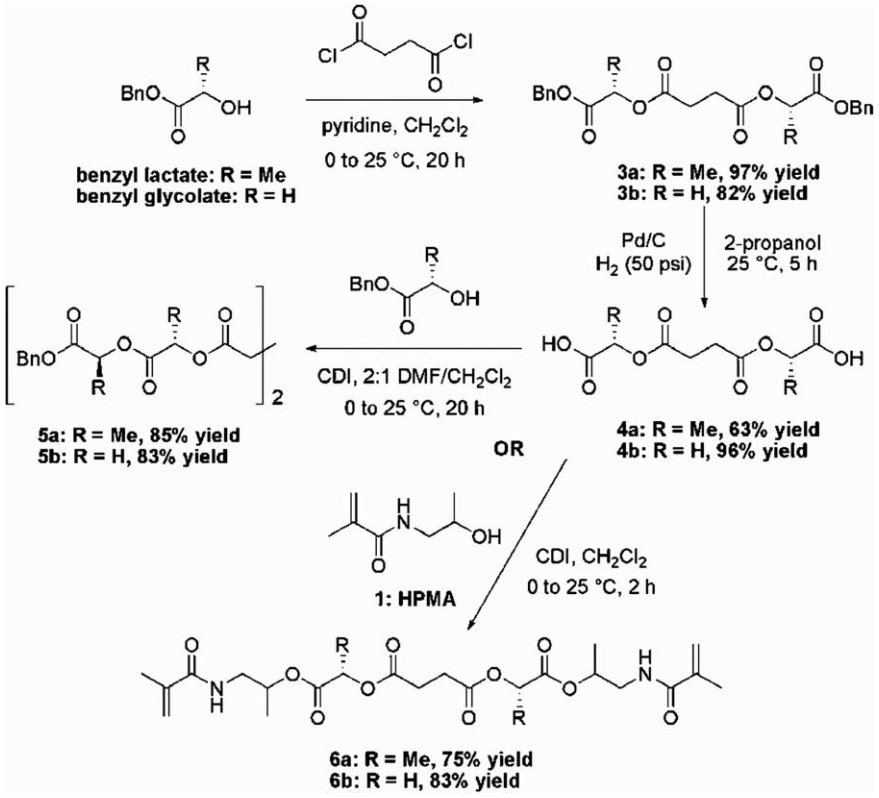
used as drug delivery devices. For our purposes, it was desirable for the crosslinker to possess the following characteristics: ease of chemical synthesis, inherent biocompatibility, and a defined molecular structure that would lead to a defined biodegradation rate. Moreover, we hypothesized that by changing the steric and electronic effects in the crosslinker, the rates of degradation of corresponding hydrogels could be predictably varied and controlled.

To that end, we designed novel crosslinkers (Fig. 2) that are composed of a symmetrical diacid (e.g., succinic acid) attached to two biodegradable regions (e.g., α-hydroxy glycolic or lactic acids monomers).[11] These biodegradable units are then terminated by a moiety (e.g., ±2-hydroxypropyl methacrylamide: HPMA) capable of crosslinking the polymer backbone. It is reasonable to assume that these materials would be biocompatible since the linear polymer poly(HPMA) is currently in clinical trials as a polymer chemotherapeutic.[12] Moreover, the component pieces of the degradable crosslinker (i.e., lactic, glycolic and succinic acids) are members of the Krebs cycle and are generally recognized as safe.

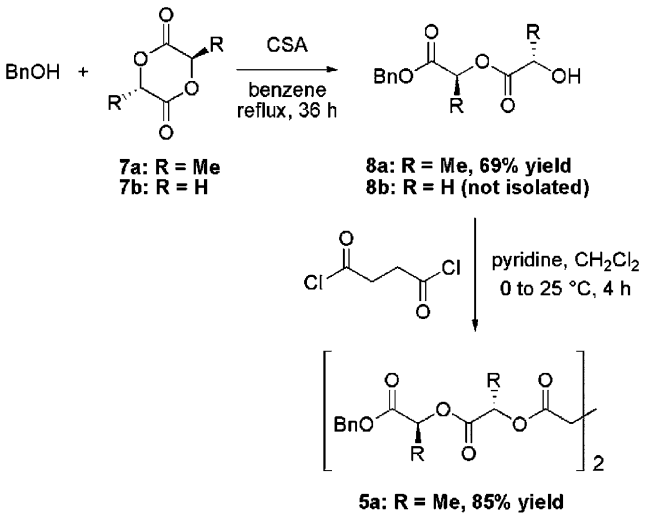
The syntheses of the crosslinkers are given in Schemes 1–4. The starting material for the first step of the synthe-



Scheme 1. Synthesis of non-degradable crosslinker **2**.



Scheme 2. Synthesis of degradable crosslinkers **6a** and **6b**.



Scheme 3. Alternate synthesis for intermediate **5a**.

sis was benzyl glycolate or benzyl lactate, which was condensed with succinyl dichloride under anhydrous conditions in the presence of 3 equiv of pyridine to give the bisbenzyl tetra ester **3** (Scheme 2).[13] The benzyl protecting group was removed from compound **3** by hydrogenolysis in 2-propanol.[14] It was found that the glycolate esters, and to a lesser extent the lactate esters, readily transesterified in the presence of primary alcohols, therefore, sterically hindered alcohols (e.g., 2-propanol) were required for this step. The resulting diacid **4** was then activated with carbonyldiimidazole (CDI) and either reacted with benzyl glycolate (or lactate) to extend the number of degradable linkages giving esters **5**, or the chain was terminated by condensation with ±2-hydroxypropyl methacrylamide (HPMA) to give **6**.[15] The synthesis of compound **2** (Scheme 1) was included because it is a homologous, non-biodegradable crosslinker and therefore could serve as a control in swelling and release studies.

A shorter and more convenient route to the construction of intermediate **5a** is given in Scheme 3. Thus, diester **8a** was prepared by an acid-catalyzed ring opening of lactide **7a** with 1 equiv of benzyl alcohol.[16] It was found that reasonable yields of **8a** could only be obtained by using a sterically hindered acid catalyst such as camphor sulfonic acid (CSA). Unfortunately, **8b** could not be isolated by distillation due to the formation of multiple oligomers. Nonetheless, **5a** was obtained in adequate yield (58% overall) from **8a** and succinyl dichloride.

Hydrogenolysis of **5b** using similar conditions for preparation of acids **4** gave intermediate **9b** in 93% yield (Scheme 4). Termination of **9b** with HPMA (**1**) using carboxyl-activating agent CDI gave the rapidly degradable crosslinker (details within) **10b** in 81% yield.

The crosslinkers were incorporated into hydrogel networks and degraded at pH 7.4, 37 °C (Fig. 3).[17] Crosslinker **6b** was incorporated in an HPMA polymer network and allowed to degrade for 2, 5 and 15 days, respectively. At pH 7, the crosslinker degraded, predominately by base hydrolysis, allowing the gel to imbibe

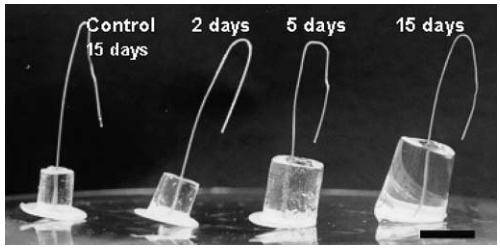
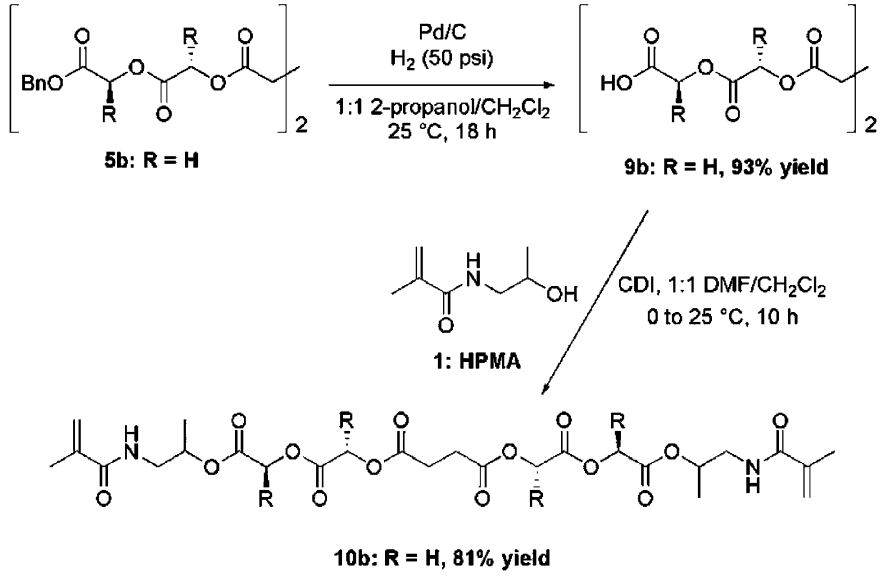


Figure 3. Photograph of the swelling and degradation profile for biodegradable hydrogels composed of 1.5 mol % crosslinker **6b** after incubation at pH 7.4 (ionic strength of 200 mM; $T = 37$ °C). From left to right: control gel made with non-degradable crosslinker **2** after 15 days, gel made with crosslinker **6b** after 2, 5 and 15 days, respectively. Scale bar: 1 cm.[11]



Scheme 4. Synthesis of degradable crosslinker **10b**.

more water and hence swell as the crosslinking density decreased. The rate of swelling could be controlled by increasing or decreasing the crosslink density or by changing the crosslinker structure. Gels made with **2** showed no discernable degradation over several months whereas gels made with **6a** degraded but more slowly (~33 days) than those made with **6b** (~13 days) or the crosslinker **10b** which hydrolyzed most rapidly (~3 days).[18] This corresponds to the expected statistical, steric, and electronic effects on the overall rate of hydrolysis at a given pH for ester oligomers. In essence, such degradation allows for a polymer gel to be transformed into a sol of linear or weakly crosslinked polymer chains, which can further degrade into completely biocompatible components.

To our knowledge, this is the first published synthesis of symmetrical, biocompatible crosslinkers with degradable regions consisting of oligoesters of α-hydroxy acids. These crosslinkers can be used to prepare biodegradable gel networks whose chemically defined crosslinks can be structurally adjusted to yield a gel that degrades at a defined rate, in response to their environment. Ultimately, these crosslinkers may be included in a wide variety of hydrophilic and hydrophobic polymer networks, which require the property of degradation. Applications include drug delivery implants, biodegradable micro- and nanogel beads, degradable water absorbents, ion-exchange resins in environmental remediation, and biodegradable cell scaffolds for tissue engineering.

Acknowledgements

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13. Preparation of **3a**: *S*-Benzyl lactate (27.0 g, 150 mmol) was stirred with pyridine (15.2 mL, 188 mmol) and succinyl chloride (8.21 mL, 75.0 mmol) in dichloromethane (100 mL) for 16 h at 25 °C. After filtering the suspension through activated carbon, the dark solution was washed with water (100 mL), 1 M HCl (2 × 50 mL), satd $NaHCO_3$ (2 × 100 mL) and brine (100 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated. Yield of **3a**: 32.3 g (97%); $[\alpha]_D$ −43.2 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.49 (d, $J = 7.1$ Hz, 6H), 2.65–2.72 (m, 4H), 5.08–5.21 (m, 6H), 7.29–7.34 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 16.6, 28.5, 66.8, 68.7, 76.5, 77.5, 127.9, 128.2, 128.4, 135.1, 170.3, 171.3. Anal. Calcd for $C_{24}H_{26}O_8$: C, 65.15; H, 5.92. Found: C, 65.06; H, 6.02.
14. Preparation of **4a**: Compound **3a** (10.2 g, 23.1 mmol) was shaken with Pd/C (1.0 g, 10 wt % Pd, Degussa type) in 2-propanol (100 mL) on a Parr hydrogenator at 50 psi H_2 at 25 °C until hydrogen uptake had ceased. Pd–C was removed by filtration through Celite, and the solvent was concentrated. Crude **4a** could be carried forward without purification, or was crystallized as its dicyclohexylamine salt from toluene/ethyl acetate/ethanol cosolvent (2:2:1). The free acid was regenerated by strong cation exchange chromatography (BioRad AG 5OW-X4, 200–400 mesh) in water/ethanol. Yield of **4a**: 3.75 g (63%); mp 59–61 °C; $[\alpha]_D$ −54.5 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.54 (d, $J = 7.1$ Hz, 6H), 2.72–2.77 (m, 4H), 5.13 (q, $J = 7.1$ Hz, 2H), 10.97 (br, 2H); ^{13}C NMR ($CDCl_3$) δ 16.6, 28.5, 68.4, 171.6, 176.3. Anal. Calcd for $C_{10}H_{14}O_8$: C, 45.81; H, 5.38. Found: C, 46.01; H, 5.55.
15. Preparation of **6a**: To compound **4a** (2.20 g, 8.30 mmol) in dichloromethane (30 mL) at 0 °C under argon CDI (2.75 g, 17.0 mmol) was added. The reaction was allowed to warm to 25 °C, and then HPMA (2.57 g, 17.9 mmol) was added. After stirring at 25 °C for 2 h, the reaction mixture was washed with 1 M NaH_2PO_4 (2 × 100 mL), satd Na_2CO_3 (10 mL), and brine (10 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated to give an oil. Yield of **6a**: 4.08 g (95%); $[\alpha]_D$ −21.3 (*c* 1.0, $CHCl_3$); ^1H 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 (DMSO-d_6, several peaks exhibited duality which may be due to hindered rotation and diastereomers) δ 16.5, 17.2, 17.4, 18.6, 28.2, 43.0, 54.9, 68.7, 70.2, 70.2, 119.1, 139.8, 139.9, 167.7, 167.8, 169.7, 169.9, 171.3, 171.4; HRMS (FAB+) Calcd for $C_{24}H_{27}N_2O_{10}$ (M+H) 513.2448, found 513.2418.
16. Preparation of **8a**: l-Lactide **7a** (15.0 g, 104 mmol) was heated to reflux with benzyl alcohol (12.4 g, 114 mmol) and CSA (139 mg, 624 μmol) in anhydrous benzene (100 mL) for 36 h. The reaction mixture was washed with 0.2 M $NaHCO_3$ (2 × 50 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was fractionally distilled under high vacuum (30 mTorr, 108–115 °C). Yield of **8a**: 19.8 g (69%); ^1H NMR ($CDCl_3$) δ 1.40 (d, $J = 6.8$ Hz, 3H), 1.49 (d, $J = 38$ Hz, 3H), 3.00 (br, ^1H), 4.28–4.38 (q, $J = 6.8$ Hz, 2H), 5.10–5.23 (m, 2H), 7.30–7.4 (m 5H); ^{13}C NMR ($CDCl_3$) 16.8, 20.3, 66.7, 66.8, 67.2, 69.3, 128.2, 128.5, 128.6, 135.1, 170.1, 175.0; HRMS (FAB+) Calcd for $C_{13}H_{16}O_5$ (M+H) 253.0998, found 253.1066.

17. Gels were formed by free radical polymerization using the ammonium persulphate/*N*,*N*,*N*′,*N*′-tetramethylethylene diamine (APS-TMED) initiation system. The gels were prepared at a mole feed ratio of 1.5 mol % crosslinker as a copolymer with 98.5 mol % HPMA **1**. An aqueous solution of HPMA **1** (2.12 g, 14.8 mmol, in 1.5 mL DI water) and crosslinker **6b** (109 mg, 0.225 mmol) were mixed and charged into 1 mL polypropylene syringes. To this mixture were added solutions of APS (166 μL of a 2.6 M solution) and TMED (204 μL of a 2.1 M solution, adjusted to pH 7 with HCl) to initiate the polymerization. The gels were allowed to polymerize for 4 h.

18. The kinetics of gel degradation were determined by weighing the gels over the course of their degradation. The gels were incubated in pH 7.4, 100 mM PBS; $I = 200$ mM at 37 °C on a temperature-controlled rotary shaker (New Brusnwick Scientific) at 30 RPM. As the crosslinker is hydrolyzed the gel network is able to imbibe more water and the gel sample increases in mass (see Fig. 3). Finally, the gel becomes very soft and is no longer capable of being weighed. Lastly, the hydrogel dissolves. For more details on degradation kinetics, see Eichenbaum, K. E. et al. *Macromolecules*, in press.