Bioerodible Hydrogels Based on Photopolymerized Poly(ethylene glycol)-*co*-poly(α-hydroxy acid) Diacrylate Macromers

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ABSTRACT: Macromers having a poly(ethylene glycol) central block, extended with oligomers of α-hydroxy acids such as oligo(*dl*-lactic acid) or oligo(glycolic acid) and terminated with acrylate groups, were synthesized and characterized with the goal of obtaining a bioerodible hydrogel that could be formed in direct contact with tissues or proteins by photopolymerization of aqueous solutions of the macromer. It was determined that the PEG component of these macromers must be greater than approximately 55 mol % to provide water solubility. The amphiphilic nature of the macromers causes them to assume a micellar conformation, which enables them to undergo rapid photopolymerization. Due to the multifunctionality of the macromers, polymerization results in the formation of cross-linked gels. These gels degrade upon hydrolysis of the oligo(α-hydroxy acid) regions into poly(ethylene glycol), the α-hydroxy acid, and oligo(acrylic acid). The degradation rates of these gels can be tailored by appropriate choice of the oligo(α-hydroxy acid) from less than 1 day to up to 4 months. Using nontoxic photoinitiators, the macromers can be rapidly photopolymerized with visible light in direct contact with tissues without excess heating or local toxicity. If polymerized in contact with tissues, the gels adhere to the tissues, presumably by interpenetration; if polymerized prior to contact with tissues, the gels are very nonadhesive, presumably by possession of a large amount of free water which is not hydrogen bonded with the ether linkages of the ethylene glycol mers. These novel materials are suitable for a number of biomedical applications and show potential for use in macromolecular drug delivery.

Introduction

Bioerosion of polymers in vivo occurs by a combination of several mechanisms. Linkages in the polymer may be labile to hydrolysis either nonenzymatically or under the influence of particular enzymes, in either case forming oligomeric scission products that may or may not be soluble in water. In the case of water-soluble oligomers, the products may be excreted directly or after entry and exit from various metabolic pathways. In the case of water-insoluble oligomers, cellular processes may be more important in bioerosion; in particular, macrophages and foreign body giant cells may take up small particles by phagocytosis, whereupon the particles are further degraded within peroxide- and enzyme-rich membrane-bound lysosomes. If the polymer is hydrophobic, then degradation is limited to the surface of the material; if the polymer is hydrophilic and water is present in the bulk of the material by virtue of its swelling, then degradation may occur throughout the bulk of the material.

Bioerodible polymers have been used extensively in medicine for structural supports[1] (sutures, staples, support meshes, etc.) and for the controlled release of drugs.[2,3] Most of the polymers used for structural supports are relatively hydrophobic and are degraded by cellular processes as a result of a local inflammatory reaction. There exists a need for materials that do not stimulate inflammation and that are degraded even in its absence. Most of the polymers used for controlled drug release are also relatively hydrophobic, so as to be degraded at the surface and thus obtain linear release kinetics, making them more useful for release of hydrophobic drugs (which are incorporated as solid solutions) rather than hydrophilic drugs, such as proteins (which are incorporated as particulate suspensions). There exists a need for hydrophilic materials that can be used to incorporate polypeptide drugs in solution.

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The most thoroughly investigated and used bioerodible polymers are the poly(α-hydroxy esters), such as poly(*dl*-lactic acid) (PLA) and poly(glycolic acid) (PGA), which were synthesized with the aim of producing polymers that would degrade into naturally occurring substances.[4-6] Recently, other materials of synthetic origin that possess hydrolyzable bonds along the polymer backbone have been developed. These include poly(anhydrides)[7] and poly(orthophosphoesters).[8] The degradation rates of these materials can be further controlled by using unsaturated multifunctional macromeric precursors and cross-linking the polymeric matrices. Domb et al. have reported poly(anhydrides) that can be cross-linked.[9] The water solubility of the scission products has been enhanced by incorporation of water-soluble polymeric blocks to produce amphiphilic polymers. Casey et al.[10] synthesized non-cross-linked BAB triblock copolymers of poly(ethylene glycol) (PEG; block A) and poly(glycolide) (PGA; block B) by using the hydroxyl end groups of the PEG segment as the ring-opening reagent. Others have synthesized similar non-cross-linked amphiphilic materials comprised of block copolymers of PEG and poly(α-hydroxy acid); these polymers absorbed up to 60% water[11] but were not water soluble.[6,11,12] Hydrogels containing enzymatically degradable azoaromatic cross-links for site-directed delivery of proteins to the colon have been synthesized by Brondsted and Kopecek.[13] Physically cross-linked hydrogels have been synthesized by rendering a water-soluble polymer water-insoluble by chemical modification, such as esterification of hyaluronic acid with monohydric alcohols[14] or alkyl chains.[15] The approach to the conversion of a water-soluble biopolymer into a cross-linked network that could undergo hydrolysis has been investigated by Welz et al.[16] using cross-linked gelatin and by others using cross-linked hyaluronic acid.[14,17,18] However, tailoring the degradation rates of such biological macromolecules is difficult, and since these biopolymeric networks are often degraded enzymatically, the rate of degradation can vary considerably with implant site.

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Hydrogels have been used extensively for controlled-release applications. Kim et al. have recently reviewed hydrogels used for drug release.[19] Most of these hydrogels, however, are nonbiodegradable, and the drug is released by diffusion.

There are relatively few systems for polymerization in vivo. The polymerization times required for these systems are quite varied. These include the cyanoacrylates[20] (a few minutes), isocyanate-terminated prepolymers[21] (tens of minutes), and methyl methacrylate based bone cements[22] (hours), in addition to biologically based materials such as fibrin glue.[23] The requirements for polymerization onto soft tissue in vivo are quite stringent, including limitations to physiologic temperatures, restriction to oxygenated and moist environments, lack of toxic organic solvents or monomers, and limitation to clinically relevant rates of polymerization (usually less than several minutes).

In this paper we detail the synthesis of nontoxic water-soluble macromers that can be photopolymerized in vivo in direct contact with tissues using long-wave ultraviolet (LWUV) or visible light to produce bioerodible polymeric hydrogels. The degradation rates and physical characteristics of these hydrogels can be tailored by appropriate choice and synthesis of the macromers. The macromers have three structural domains: a water-soluble polymer as the central domain; hydrolytically labile oligomeric extensions at each end of the central water-soluble domain; and photopolymerizable end groups that terminate the oligomeric extensions. When such a macromer is cross-linked through the polymerizable end groups, it should degrade (become soluble) when one chain scission occurs on each of the two extensions. With proper choice of all parts of the macromer, it is possible to design a series of macromers with a high degree of control over the final properties of the cross-linked gel. We chose PEG as the central water-soluble domain because of its unique biocompatibility and polymerization characteristics. PEG is soluble in water and is readily cleared by the body.[24] When immobilized either chemically[25,26] or physically[27] on polymer surfaces, it renders the surface highly resistant to biological fouling. This includes reduction in protein adsorption and resistance to bacterial and animal cell adhesion.[27] PEG is apparently not readily recognized by the immune system; modification of proteins with PEG has been shown by Fuerteges et al. to reduce immunogenicity and antigenicity of these proteins and to increase circulation times.[28] The nontoxicity and micelle-forming properties of PEG have been exploited by Yokoyama et al. to reduce the toxicity and enhance in vivo antitumor activity of adriamycin.[29] PEG modified with polymerizable end groups polymerizes at a much higher rate in water, over that in organic solvents, because of a micellar nature of the macromer in aqueous solution.[30] The hydrolytically labile extensions were chosen to be a poly(α-hydroxy acid), such as PGA or PLA, because the in vitro and in vivo degradation of these polymers is well understood and the degradation products are natural metabolites that are readily eliminated by the body.[31] The molecular weight of the hydrolytically labile oligomeric extension was chosen, relative to that of the water-soluble central domain, to be sufficiently small that the properties of the macromer in solution, and the gel properties, were determined primarily by the central water-soluble chain. Acrylates were chosen as end groups because they undergo very rapid photopolymerization, especially with visible light, when used with the proper initiating system;[32] methacrylates could similarly be used. We have recently reported the use of

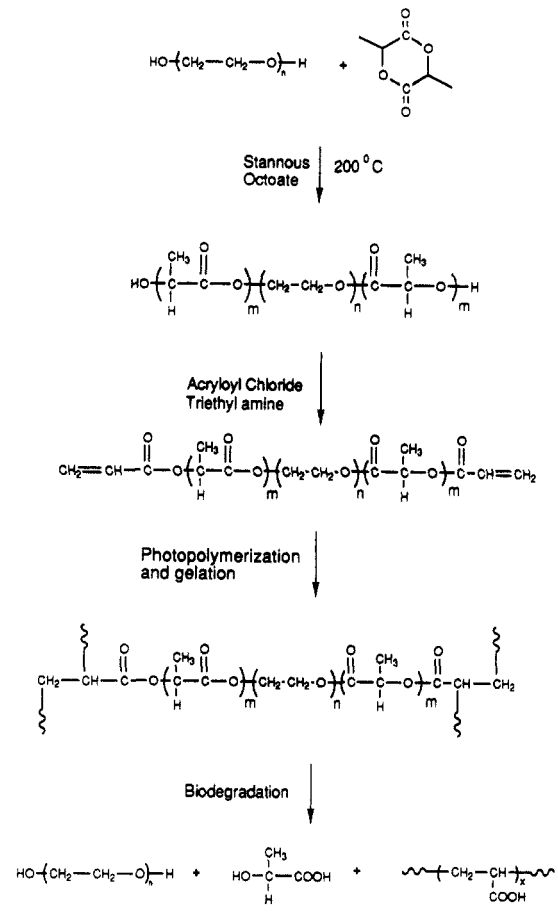

Figure 1. Reaction scheme for the synthesis of polymerizable PEG-*co*-poly(α-hydroxy acid) di- and tetraacrylates and hydrogels, as well as their degradation.

rapid visible laser-induced photopolymerization of PEG di- and tetraacrylates for the encapsulation of animal cells.[33] Also, one of the degradation products, in addition to PEG and the α-hydroxy acid, is expected to be a small amount of oligo(acrylic acid) (degree of polymerization of 2 or 3), which is water soluble and relatively nontoxic.[34] The overall reaction scheme is shown in Figure 1.

Materials and Methods

Materials. PEGs with molecular weights 1000 (PEG 1K), 4000 (PEG 4K), 6000 (PEG 6K), and 20 000 (PEG 20K) were obtained from Fluka. PEGs with molecular weights 10 000 (PEG 10K) and 18 500 (PEG 18.5K) were purchased from Aldrich and Polysciences Inc., respectively. All PEGs were α,ω-dihydroxy, with the exception of PEG 18.5K. The PEG 18.5K was prepared (by Polysciences) by coupling two α,ω-dihydroxy poly(ethylene glycol) (MW 9000) chains with a Bisphenol A bisepoxide, yielding four hydroxy groups per chain (one α, one ω, and two on the Bisphenol A linker). All PEGs were dried by azeotropic distillation with benzene. *dl*-Lactide and glycolide were obtained from Aldrich and E. I. du Pont de Nemours, respectively, and were recrystallized from ethyl acetate prior to use. The LWUV initiator 2,2-dimethoxy-2-phenylacetophenone and the visible light initiator ethyl eosin were obtained from Aldrich. All other chemicals used were of reagent grade and were used without further purification.

Synthesis of PEG-*co*-poly(α-hydroxy acid) Copolymers.[35,36] Several PEG-*co*-poly(α-hydroxy acid) copolymers were synthesized; the synthesis of a copolymer of PEG 6K with *dl*-lactide is illustrated below. A total of 30 g of dry PEG 6K, 3.60 g of *dl*-lactide (5 mol *dl*-lactide/mol of PEG), and 15 mg of stannous octoate were charged into a 100-mL round-bottomed flask under a nitrogen atmosphere. The reaction mixture was stirred under vacuum at 200 °C for 4 h and at 160 °C for 2 h and was subsequently cooled to room temperature. The resulting copolymer was dissolved in dichloromethane, precipitated in

anhydrous ether, filtered, and dried (yield 95%). The α- and ω-hydroxyl end groups of PEGs with various molecular weights were used as ring-opening reagents to initiate the polymerization of either *dl*-lactide or glycolide to similarly form several other copolymers.

Synthesis and Characterization of PEG Macromers. The above-described PEG-*co*-poly(α-hydroxy acid) copolymers, which were themselves α- and ω-terminated by hydroxyl groups, were end-capped with acrylate groups to form a polymerizable macromer; the synthesis of the macromer with the PEG 6K is illustrated. A total of 30 g of the above-described intermediate copolymer was dissolved in 300 mL of dichloromethane in a 500-mL round-bottomed flask and was cooled to 0 °C in an ice bath. A total of 1.31 mL of triethylamine and 1.58 mL of acryloyl chloride were added to the flask, and the reaction mixture was stirred for 12 h at 0 °C and 12 h at room temperature. The reaction mixture was filtered to remove triethanolamine hydrochloride, and the macromer was obtained by pouring the filtrate in a large excess of dry diethyl ether. It was further purified by dissolution and reprecipitation once using dichloromethane and hexane, respectively. Finally, it was dried at 70 °C under vacuum for 1 day. This PEG macromer was called 6KL5. This indicates that the acrylate-terminated macromer was synthesized from PEG segments of molecular weight 6000 (*6K*L5), using *dl*-lactide as the extension of the PEG α- and ω-hydroxy end groups (6K*L*5), and the degree of polymerization of the lactoyl repeats (not lactidyl repeats, the cyclic dimer of lactic acid, which is actually polymerized) was 5 per hydroxy end group (6KL*5*). Several other macromers were similarly synthesized by terminating PEG-*co*-poly(α-hydroxy acid) copolymers with acrylate or methacrylate groups. Specifically, the letter G in the nomenclature denotes macromer utilizing glycolide as the α-hydroxy acid cyclic dimer.

IR spectra were measured using a Digilab FTS 15/90 IR spectrometer. The 300-MHz proton NMR spectra were measured with a Nicolet NT-360 spectrometer; spectra were recorded in $CDCl_3$, and tetramethylsilane (TMS) was used as internal standard.

UV Polymerization. A 23% w/v solution of macromer in phosphate-buffered saline (PBS: pH 7.4, 0.2 g/L of KCl, 0.2 g/L of KH_2PO_4, 8 g/L of NaCl, 1.15 g/L of Na_2HPO_4) was used. To 1 mL of this solution was added 3 μL of the initiator solution (300 mg of 2,2-dimethoxy-2-phenylacetophenone dissolved in 1 mL of *N*-vinylpyrrolidinone). A total of 0.1 mL of this macromer solution was poured onto an 18 × 18 mm glass coverslip and irradiated using a low-intensity, portable, LWUV lamp (Blak-Ray Model 3-100A with Flood 365 nm, 8 mW/cm²) until gelation occurred. The time required to induce nontacky gelation was noted. This was accomplished by continuously scratching a film of the macromer solution with a sharp instrument until the scratched film was seen to retain the scratch mark.

Visible Laser Polymerization. A 23% w/v solution of macromer in PBS with ethyl eosin (0.5 mM) and triethanolamine (0.5 M) was irradiated with an argon ion laser (American argon-ion laser Model 905 emitting at 514 nm) at a power density of 7 W/cm². The beam was expanded to a diameter of 3 mm, and the sample was slowly scanned until gelation occurred.

Degree of Gelation and Water Content. Solutions of various degradable macromers were made as described above. Gels in the shape of disks were made using a mold. A total of 400 μL of solution was used for each disk. The solutions were irradiated for 2 min to ensure thorough gelation. The disk-shaped gels were removed and dried under vacuum at 60 °C for 2 days. The disks were weighed (W1) and then extracted repeatedly with chloroform for 1 day. The disks were dried again and weighed (W2). The gel fraction was calculated as W2/W1.

Subsequent to extraction, the disks were allowed to equilibrate with HEPES-buffered saline (HBS: 0.9% NaCl, 10 mM *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid)) for 6 h and weighed (W3) after excess water had been carefully swabbed away. The total water content was calculated as W = (W3 − W2)/W3 × 100%. Differential scanning calorimetry (DSC; Perkin-Elmer DSC-7) was used to determine the amount of free water that was not bound by hydrogen bonding with the ethylene glycol repeat units of the PEG central domain. Gels equilibrated with HBS were carefully swabbed with filter paper to remove excess water before measurement. A scan rate of 20 °C/min was used, and the heat capacity for the endotherm for water melting between 0 and 6 °C was measured (H1). The heat capacity of a sample of HBS was also measured (H2). The fraction of free water was calculated as H1/H2. The residual water (difference between the total water and the free water) was assumed to be bound due to hydrogen bonding with the PEG segments. The number of moles of this bound water per mole of ethylene glycol repeat unit was calculated as $N = W \times 44/(18 \times (100 - W))$.

In Vitro Degradation. A total of 200 mg of polymer solution in PBS was polymerized using the LWUV lamp in the shape of disks using a plastic mold. After polymerization, samples were dried in a vacuum oven at 70 °C for 24 h and then extracted with chloroform repeatedly, for 1 day to remove unreacted macromer. The samples were redried and equilibrated in PBS at pH 7.3 and incubated at 37 °C. Weight loss was monitored gravimetrically at various intervals of time.

In Vitro Fibroblast Adhesion and Spreading. The ability of these gels to resist cell adhesion was determined in vitro by seeding human foreskin fibroblasts (HFF) onto the surface of the gels. HFF cells were obtained in house from neonatal foreskins and were routinely grown in Dulbecco's modification of Eagle's medium, supplemented with 10% fetal calf serum (Gibco) and 1% antibiotic/antimycotic solution (Gibco) at 37 °C in a 5% CO_2 atmosphere. A total of 0.2 mL of sterile macromer solution was gelled on 18 × 18 mm glass coverslips using the LWUV lamp. The gels were washed with PBS and seeded with HFF cells at a density of 30 000 cells/cm². A Coulter counter (Coulter Electronics) was used to determine cell density. The extent and morphology of cell attachment of the cells to the gel surfaces were monitored every 2 h, and after 6 h the number of attached cells and their morphology were recorded and quantified using phase-contrast light microscopy. At least five different fields, at predetermined locations, were counted to determine the number of adherent cells.

Controlled Release of Albumin. A total of 1 g of 1KG2 was mixed with 200 μL of bovine serum albumin solution (20 mg/mL in PBS) and 2,2-dimethoxy-2-phenylacetophenone. The mixture was charged into a 3-mm-diameter glass test tube and exposed to the LWUV lamp for 2 min. The gel was removed from the tube and sliced into 2-mm-thick disks. These disks were kept in 10 mL of PBS at 37 °C, and the concentration of albumin released was monitored using a Biorad total protein assay reagent.

Results and Discussion

Synthesis and Polymerization. Telechelic oligomers having hydroxyl end groups terminating oligo(lactidyl), oligo(glycolidyl), or oligo(caproyl) moieties were obtained by ring opening of the cyclic monomer or dimer by the α- and ω-hydroxyl end groups in bulk at 200 °C using appropriate amounts of lactide, glycolide, or ε-caprolactone and PEG in the presence of stannous octoate as a transesterification catalyst.[35,36] The reaction sequence is shown in Figure 1. The ring-opening polymerization of lactones can be initiated by an alcohol using a variety of catalysts.[34,35] The propagation reaction consists of stepwise addition of the lactone to the terminal hydroxyl groups. The use of PEG diol initiator results in a BAB copolymer having terminal hydroxyl groups.[36] The degree of polymerization depends upon the ratio of lactone concentration to alcohol concentration. Several polymers were synthesized using a variety of PEG chains as ring-opening reagents and are shown in Table I. The FTIR spectrum of a typical polymer, 18.5KG2, along with that of its PEG precursor, is shown in Figure 2. The FTIR spectra of the PEG precursor showed an absorption band at 3510 cm^{-1} due to the terminal hydroxyl group. This band disappeared in the 18.5KG2 due to acrylation. The bands at 2880 cm^{-1} and at 1110 cm^{-1} were attributed to the C–H stretch and ether stretch, respectively[34,36] and were present in both the polymers. A strong absorbance was seen at 1756 cm^{-1} in the 18.5KG2 which confirmed the presence of the ester stretch due to the glycolidyl moieties. The degree of polymerization (DP) of the lactide

Table I
Synthesis and Characteristics of Macromers

polymer code	PEG mol wt	monomer	DP of polyester	appearance	polyester DP as % of PEG DP[a]	solubility in water[b]
1KL2	1000	lactide	2	viscous liquid	17.6	soluble
1KL5	1000	lactide	5	viscous liquid	44	insoluble
1KL10	1000	lactide	10	viscous liquid	88	insoluble
1KL15	1000	lactide	15	soft solid	132	insoluble
1KL30	1000	lactide	30	soft solid	264	insoluble
1KL40	1000	lactide	40	solid	352	insoluble
6KL5	6000	lactide	5	solid	7.3	soluble
10KL5	10000	lactide	5	solid	4.4	soluble
18.5KL2	18500	lactide	2.5	solid	2.4	soluble
20KL10	20000	lactide	10	solid	4.4	soluble
1KG2	1000	glycolide	2	viscous liquid	17.6	soluble
4KG5	4000	glycolide	5	solid	11.0	soluble
6KG5	6000	glycolide	5	solid	7.3	soluble
10KG5	10000	glycolide	5	solid	4.4	soluble
18.5KG2	18500	glycolide	2.5	solid	2.4	soluble
20KG15	20000	glycolide	15	solid	6.6	soluble
0.2KD[c]	200			viscous liquid	44	insoluble
0.4KD[c]	400			viscous liquid	22	soluble

[a] DP of the repeating α-hydroxy acid, not of the cyclic dimer which is actually polymerized. [b] Solubility evaluated for PEG-*co*-poly(α-hydroxy acid) or for PEG diacrylates (last two rows). [c] PEG diacrylates; no α-hydroxy acid comonomers.

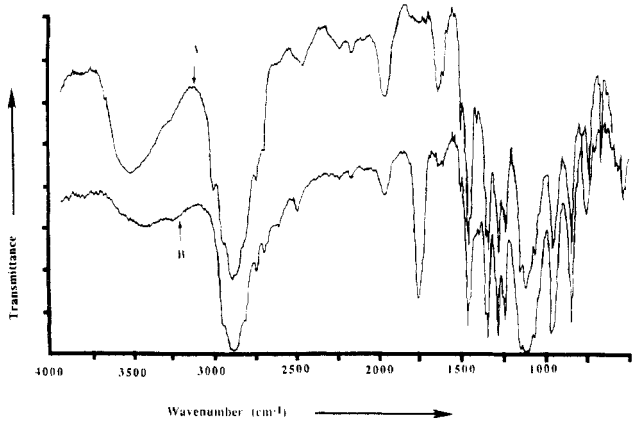

Figure 2. FTIR spectrum of PEG (MW 18 500), the poly(ethylene glycol) precursor (A) and 18.5KG2 (B), the copolymer of PEG (18 500) with glycolic acid and terminated with acrylate groups. A strong absorption at 3510 cm^{-1} in the PEG precursor due to the terminal hydroxyl group can be seen. This band is absent in the spectrum of 18.5KG2 due to acrylation. A strong absorbance at 1756 cm^{-1} in the 18.5KG2 confirms the presence of the ester stretch due to the glycolidyl moieties.

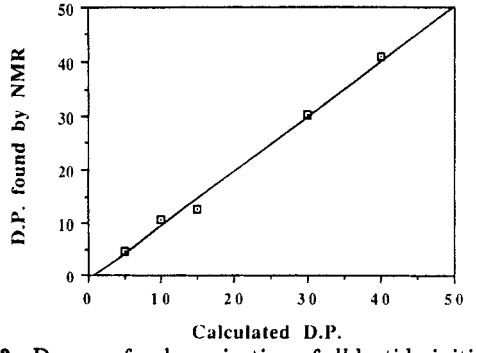

Figure 3. Degree of polymerization of *dl*-lactide initiated by ring opening by PEG (MW 1000) α- and ω-hydroxyl terminal groups as measured by ^1H NMR and calculated stoichiometrically. The linear relationship indicates that the reaction proceeds to completion under these conditions.

in the lactide copolymers was calculated using integral NMR intensities of $-CH_3$ (1.3 ppm) from lactide and $-CH_2O-$ (3.63 ppm) from PEG. These assignments were determined by comparison with spectra for the corresponding homopolymers and use of standard literature values. The calculated DP was plotted against the ratio of monomer/PEG ([M]/C) and is shown in Figure 3. A linear relationship was found, in agreement with the predicted polymerization kinetics.[37]

Table II
Photopolymerization Gel Times and Conversions of Various Macromers

polymer	UV initiated (s)[a,b]	gel content (%)[b]	polymer	UV initiated (s)[a,b]	gel content (%)[b]
1KG2	53.0 ± 4.1	61.3 ± 5.2	20KG10	13.3 ± 0.5	47.0 ± 4.9
4KG5	14.7 ± 0.5	56.3 ± 0.9	6KL5	21 ± 0.8	73.8 ± 9.5
6KG5	9.3 ± 0.5	66.5 ± 2.4	10KL5	7.7 ± 0.5	67.9 ± 15.4
10KG5	18.0 ± 0.8	54.3 ± 0.6	18.5KL2	13.9 ± 0.7	64.8 ± 9.0
18.5KG2	23.3 ± 1.2	44.5 ± 4.8	20KL10	16.5 ± 0.6	66.7 ± 6.2

[a] 2,2-Dimethoxy-2-phenylacetophenone, 900 ppm, 0.1 mL of 23% (w/v) monomer solution (in *N*-vinylpyrrolidinone) in PBS at pH 7.3, 25 °C. [b] Mean ± SD (n = 5).

The solubilities of these copolymers in water are shown in Table I. One objective of this study was to obtain water-soluble macromers. PGA and PLA are insoluble in water. As the mass fraction of the poly(α-hydroxy acid) in the PEG-*co*-poly(α-hydroxy acid) copolymer increases, its solubility in water decreases. It is interesting to note that when the ratio of the DP of the polyester to the DP of the PEG is less than about 0.45, the copolymer is soluble in water. Thus, we sought to maintain the DP of the polyester extension less than about 45% of the DP of the PEG central water-soluble domain in the polymers we investigated further.

The terminal hydroxyl groups in the PEG-*co*-poly(α-hydroxy acid) precursor were subsequently converted to acrylate groups by a reaction with acryloyl chloride. Since PEG has two hydroxyl groups per molecule (except for the tetrahydroxypoly(ethylene glycol) 18 500), the number of acrylic groups upon the hydroxyl-initiated oligo(α-hydroxy acid) extension is expected to be 2 (or 4 for the PEG 18 500). Thus, upon free-radical polymerization, these macromers form a cross-linked three-dimensional gel. The gelation times for several macromers under physiological conditions (in PBS, pH 7.3) under LWUV initiation are reported in Table II.

It may be observed that these monomers undergo very rapid photopolymerization, even in the presence of oxygen; the LWUV gelation times ranged from 5 to 53 s. To achieve gelation and water insolubility, a three-dimensionally cross-linked network with an essentially infinite molecular

weight must form. The low molecular weight precursors must undergo a higher number of reactive couplings to achieve this state and thus gel somewhat slower than the higher molecular weight precursors. Moreover, precursors having a molecular weight below 4000 may be unable to form a stable micellar configuration, thus further contributing to slower gelation. The optimal molecular weight for micelle formation by PEG chains was determined to be between 5000 and 10 000.[30] Thus, no added benefit in gelation rate was obtained for higher molecular weight precursors. These polymerization times were obtained using an initiator concentration of 900 ppm and a low-intensity portable LWUV lamp. A low concentration of the initiator is desirable to minimize the presence of substances whose nontoxicity in vivo has not been thoroughly established. Using higher initiator concentrations, up to a 4-fold increase in the rate of polymerization of these macromers could be obtained. An increase in the intensity of the LWUV light would also enhance the rates of polymerization further, since the polymerization rate increases in direct proportion to the square root of the light intensity. Photopolymerization initiated by visible light, using the argon-ion laser (514 nm), was extremely rapid and was complete in less than 1 s under our experimental conditions (7 W/cm^2) using each of the precursors examined.

These gelations were accomplished in water under physiological conditions and in the presence of oxygen. Oxygen is usually an inhibitor of free-radical polymerizations. As described above, when PEG is end capped with hydrophobic polymerizable units, it forms micellar structures in water.[30] This micellar structure orients and raises the effective concentration of double bond within the micelle, which leads to an increased rate of the propagation reaction in a free-radical polymerization. The radical termination reactions are diffusion controlled and are retarded by an increase in the viscosity of the medium, which restricts the segmental motion of the involved polymer radical. Thus, an increase in the propagation rate and a decrease in the termination rate results in a high polymerization rate[30] and rapid gelation. The rate of photopolymerization increases with the square root of the light intensity at both low and high extents of conversion. With use of the argon-ion laser, the reaction rates can be enhanced by using a high-intensity noncytotoxic and nonmutagenic visible wavelength.[32] Thus, a combination of reactive micelle-forming macromers in water and a rapid photoinitiating system using an argon-ion laser provides an attractive technique for the polymerization of these macromers in vivo.

The liberation of heat during polymerization reactions in vivo may present limitations on their use. Since the monomers are macromers, the number of vinyl bonds per unit mass is very small, leading to very low heats of polymerization. This is particularly important if polymerization is to be carried out in contact with living tissues, since the liberation of excessive heat can lead to tissue necrosis and scar formation.

The extents of conversion of the various macromers, after prolonged exposure to the appropriate light, are shown in Table II. The gelling fraction of the precursor solution ranged from about 44% to about 74%. Conversion was least in macromers of high DP with less hydrophobic end groups (glycolide), which may reflect a lesser tendency to form micellar structures at their end groups.

Gel Characterization. The photopolymerization of the water-soluble macromers results in the formation of highly hydrated gels. The water contents of various photopolymerized hydrogels are shown in Table III, as determined gravimetrically. DSC was used to discriminate between free and bound water in these gels, and a typical thermogram for an 18.5KG2 gel appears in Figure 4. The thermogram shows an endothermic peak between 0 and 7 °C; this peak was assigned to free water associated with the PEG gel and was used to calculate the amount of free water present in these gels.[38] The bound water was calculated from the differences between the free water and total water content. The number of water molecules associated with each oxyethylene unit of PEG was found in the range of 2–3 for most gels, which is in accordance with reported values in literature.[38] A broad melting peak for PEG and the glycolidyl moieties appears from about 65–130 °C. The cross-linked nature of the PEG segments prevents a sharp melting transition. The 10KG5 and the 20KG10 gels partially degraded within 1 day and upon equilibration were already significantly degraded. Upon swabbing with filter paper, these gels became partially dehydrated and thus gave unnaturally low free water contents. Thus, the amount of bound water indicated for these gels may be overestimated. This may have occurred to a lesser extent in the 10KG5 gel as well.

Table III
Water Contents of PEG Networks

polymer code	water absorption (%)[a]	free water (%)[b]	bound water (%)[c]	mol of water/mol of ethylene glycol
1KG2	82.3 ± 2.6	68.4	13.9	1.9
4KG5	87.3 ± 1.8	78.0	9.3	1.8
6KG5	88.1 ± 3.3	74.8	13.3	2.8
10KG5	94.5 ± 0.5	83.7	10.8	4.8[d]
10KL5	91.7 ± 0.5	82.0	9.7	2.8
18.5KG2	89.8 ± 0.4	81.4	8.4	2.0
20KG10	94.5 ± 0.4	79.8	14.7	6.6[d]

[a] Determined gravimetrically, mean ± SD (n = 5). [b] Determined by differential scanning calorimetry. [c] Determined by difference. [d] Accurate determination of the total water was not possible due to rapid degradation of gels in vitro; thus, the fraction of bound water and the number of water molecules per oxyethylene group are higher than actual.

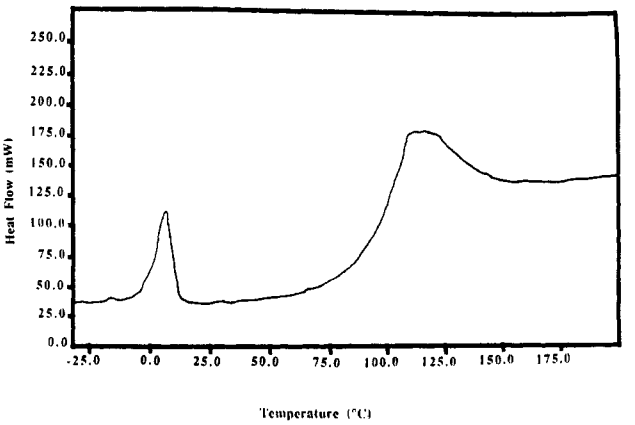

Figure 4. Differential scanning calorimetry thermogram for the hydrated 18.5KG2 gel formed by polymerization with LWUV light.

Degradation. The degradation characteristics of these gels are expected to be generally similar to those of PLA or PGA,[39-41] with the most notable difference being bulk (for the gels) rather than surface (for the relatively hydrophobic PLA or PGA homopolymers) hydrolysis. The cross-linked PEG-*co*-polyesters were subjected to in vitro hydrolysis. Figure 5 shows degradation profiles of two representative cross-linked gels made from precursors 4KG5 and 20KL10. The total time taken to degrade the

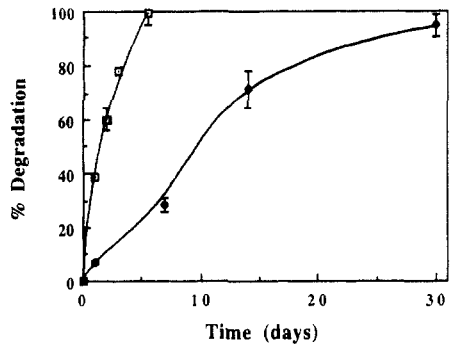

Figure 5. Degradation kinetics for the 4KG5 (◆) and 20KL10 (□) hydrogels formed by polymerization with LWUV light. Degradation was in HEPES-buffered saline, pH 7.3.

Table IV
In Vitro Degradation Times of PEG-*co*-poly(α-hydroxy acid) Networks in PBS (pH 7.3, 37 °C)

polymer code	time for total degradation (days)	polymer code	time for total degradation (days)
1KG2	45	1KL2	65
4KG5	6	6KL5	40
6KG5	5	10KL5	50
10KG5	1	18.5KL2	120
18.5KG2	25	20KL10	35
20KG10	0.7		

various gels into completely water-soluble products at pH 7.3 and 37 °C is shown in Table IV. These degradation times varied from 2 days to 4 months, depending on cross-linking density and the type of polyester used as the terminal block on the central PEG segment. Gels made from lower molecular weight precursor were more tightly cross-linked and thus degraded more slowly than gels made from higher molecular weight precursors. Thus, the 1KG2 gels took 45 days to degrade, while the 20KG10 gels degraded within 1 day. This was particularly evident in precursors having the same glycolidyl DP. Keeping the glycolidyl DP constant and changing the PEG molecular weight from 4000 (in 4KG5) to 10 000 (in 10KG5) enhanced the rate of degradation from 6 days to 1 day. As seen from Figure 1, two scission (hydrolysis) events per cross-linked macromer chain will result in that polymer chain entering into solution in the aqueous surroundings. The degradation of the gel is a function of the cross-link density, as well as the hydrolytic susceptibility of polyester. The in vivo and in vitro degradation characteristics of PLA and PGA are well documented.[39-41] These polyesters degrade by hydrolysis of the ester linkage. The times required for degradation of non-cross-linked PEG-containing copolymers have been reported recently, and it was shown that the presence of PEG reduces degradation times.[36] This was attributed to the hydrophilic nature of PEG,[36,38] which solubilizes larger molecular weight fragments than would be soluble for the homopolyester itself. Also, PEG increases the accessibility of water to the polymer matrix. This ensures bulk degradation of the gels. The hydrolytic susceptibility of the ester linkages is in the following order: glycolidyl > lactoyl > ε-caprolactyl (quantitative results not reported). Similar behavior has been reported for the homopolymer polyesters.[42] In vivo degradation studies and the analysis of degradation products are in progress. The in vivo degradation products are presumably PEG, monomeric and oligomeric α-hydroxy acid, and oligomeric acrylic acid (DP 2 or 3).

Biological Properties. The in vitro response of HFF cells to photopolymerized gels was evaluated through cell cultures on the polymeric networks. The number of cells attached to representative samples, along with those for

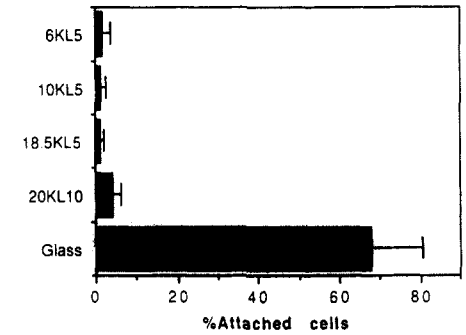

Figure 6. Attachment of human foreskin fibroblasts to various PEG-*co*-poly(α-hydroxy acid) di- and tetraacrylate hydrogel formulations in DMEM medium containing 10% fetal calf serum.

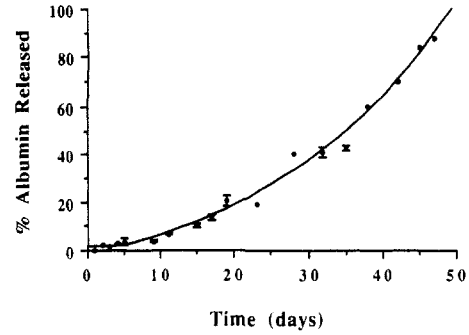

Figure 7. Release of bovine serum albumin from a 1KG2 gel as measured by the total protein release.

a glass control surface, is plotted in Figure 6. It may be observed that cell adhesion is significantly retarded on the PEG-based hydrogels. Hydrated PEG chains have been previously demonstrated to be highly resistant to protein adsorption and cell adhesion.[43] This resistance of adsorption is attributed to the hydrophilicity and high segmental mobility of PEG in water. Cell attachment and growth is mediated through the interaction of cell-surface receptors with adsorbed proteins.[44] The highly motile PEG chains exert a volume exclusion and steric repulsion effect on adsorbing macromolecules and thus reduce protein adsorption and cell adhesion.

Controlled Release of Albumin. Protein was entrapped within these bioerodible hydrogels, and the gels were used to release the proteins in a sustained and controlled manner. The permeability of the gels to protein depends upon the cross-linking density of the gel and upon the molecular weight of the protein. In highly permeable gels, made with high molecular weight PEG or releasing low molecular weight protein, release may precede degradation. In less permeable gels, as degradation occurs, permeability increases, and the rate of release correspondingly increases. The release of bovine serum albumin from 1KG2 is shown in Figure 7. A continuous release of albumin for a period of 2 months was achieved using this polymer. The rate of release is slow initially and increases somewhat as the hydrolysis occurs. During initial degradation, the release is slow since the gel is impermeable to albumin, but, as degradation continues, it leads to reduction in cross-linking density, higher water uptake, and greater porosity, which results in an increased rate of release.[45,46] Release from exclusively surface-bioeroded materials is expected to display zero-order kinetics, and that from bulk-bioeroded materials is expected to display nearly first-order kinetics. The release of protein from this particular gel, and from other gels within this family of gels, is not first order, as might be expected. The release does not have a square-root dependence, as is the case for release of the active agent due to diffusion from a hydrogel. The decrease in protein concentration within the gel matrix

(i.e., the lower driving force for diffusion out of the gel) is somewhat offset by the increased swelling and pore sizes in the gel (i.e., decreased resistance to diffusion out of the gel). This results in an enhanced rate of release.

Conclusions

Water-soluble PEG-based macromers were synthesized by initiating the polymerization of cyclic lactones using PEG α- and ω-hydroxyl terminal groups and were subsequently end capped with acryloyl groups. These materials undergo rapid polymerization and gelation under mild conditions. The physical properties and degradation rates can be controlled by choosing appropriate PEG molecular weight, hydrolyzable comonomers and end groups. Other α-hydroxy acids and their lactones such as ϵ-caprolactone, δ-valerolactone, and γ-butyrolactone can also be used as the hydrolyzable extenders of the PEG chains. These gels were found to be highly cell nonadhesive. Our experimentation, reported elsewhere,[46] has indicated that the gels adhere well to tissues when polymerized from liquid in direct contact with the tissues, presumably by forming an interpenetrating network with the extracellular proteins in the tissues. These materials also appear useful in the sustained release of proteins. A continuous release of albumin up to 2 months was achieved using gels based on water-soluble PEG-*co*-poly(*dl*-lactide) precursors, and longer durations are possible with PEG-*co*-poly(ϵ-caprolactone) precursors.

The ability of these gels to conformally adhere to tissue makes them potentially suitable for burn dressings, surgical adhesives, and modulators of tissue interactions with other tissues. We have investigated the use of these gels in the prevention of postoperative adhesions.[46] These photopolymerized gels showed remarkable efficacy in the formation of a closely conforming barrier on surgically traumatized organs, which prevented the organ from forming scar adhesions to other organs in the pelvic cavity, while permitting it to heal without appreciable scar formation.

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