Synthesis and Characterization of a Redox-Initiated, Injectable, Biodegradable Hydrogel

Wen Zhu, Jiandong Ding

Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science, Fudan University, Shanghai, 200433, China

Received 6 January 2005; accepted 8 May 2005
DOI 10.1002/app.22674
Published online 12 December 2005 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A chemically crosslinked biodegradable hydrogel was prepared via a macromer technique, and physicochemical characterizations associated with its potential application as an injectable biomaterial were carried out. The macromers were composed of poly(ethylene glycol) extended with oligomers of biodegradable polyesters such as oligolactide and end-capped with acryloyl groups. Hydrogels were obtained through the polymerization of the macromer aqueous solutions in phosphate-buffered saline initiated by a redox initiator system at body temperature. The initiator system was composed of ammonium persulfate as an initiator and *N,N,N′,N′*-tetramethylethylene diamine as an accelerator. The modulus of this chemical gel was much higher than that of a Pluronic physical gel. In vitro biodegradation was also confirmed. The degradation rates were highly tunable by the adjustment of several factors, such as the kind of ester group, the block length of the oligoester, and even the concentration of the accelerator used in the crosslinking reaction. The gelation time could be adjusted to meet the requirements of an injectable biomaterial. The effect of the polymerization heat seemed not to be significant. This kind of biodegradable hydrogel might be in situ formed after being injected into the body and shows potential applications as a unique tissue engineering material free of porogening techniques in scaffold fabrication and less invasive in implantation. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 99: 2375–2383, 2006

Key words: biodegradable; hydrogels

INTRODUCTION

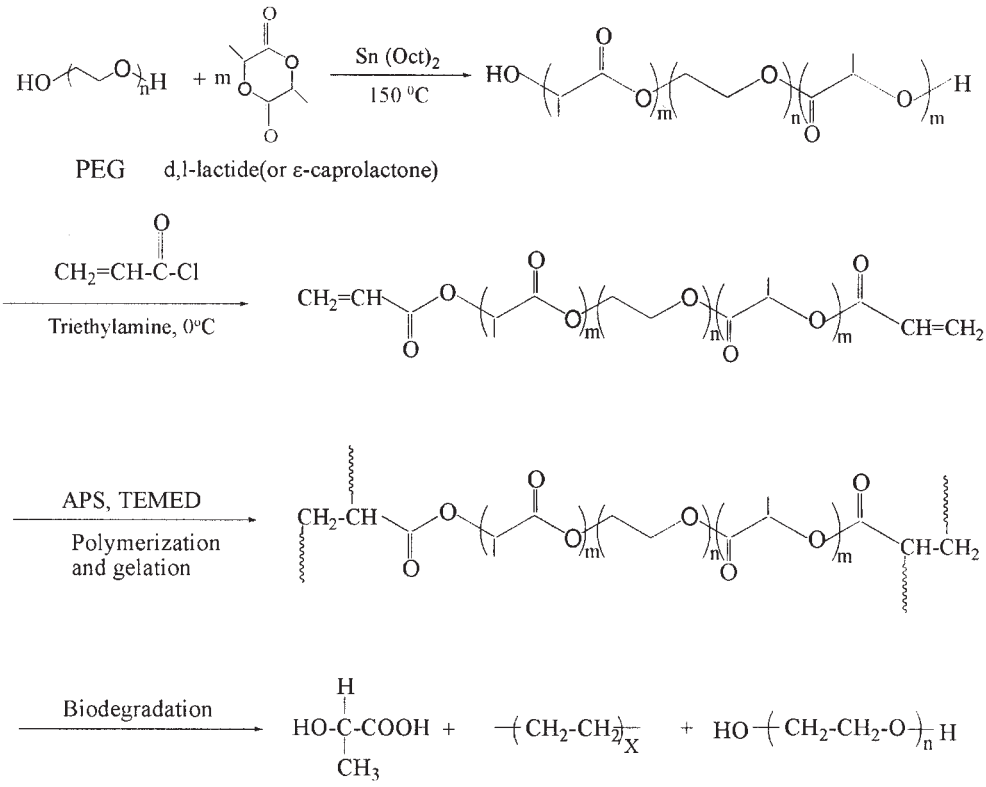
Hydrogels exhibit excellent biocompatibility and hydrophilicity[1–3] and closely resemble natural living tissues because of their high water content and rubbery consistency. The hydrogels based on poly(ethylene glycol) (PEG) and its derivatives or copolymers have been paid much attention in medical fields.[4–12]

In recent years, tissue engineering has emerged as a hot topic for developing biological substitutes that restore, maintain, or improve the lost or damaged tissues and organs.[13] Generally, biodegradable materials are used as temporary support matrices for cell transplantation. The most widely investigated biodegradable polymers as tissue engineering scaffolds are some poly(hydroxy ester)s such as polyglycolide, polylactide, poly(ε-caprolactone), and their copolymers,[14–18] which are degraded by the hydrolysis of the ester linkage into naturally occurring substances. These synthesized polymers should be prefabricated with a certain shape and with three-dimensional, porous structures.[14]

Although most tissue engineering scaffolds are necessarily porous foams, injectable biomaterials may, in some circumstances, be more suitable for treating irregularly shaped defects without heavy loading. An injectable tissue engineering hydrogel distinguishes itself because it is free of porogening techniques in scaffold fabrication and is less invasive in implantation.

A typical injectable scaffolding strategy is to employ Pluronic, a poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO) triblock copolymer,[6] an aqueous solution of which exhibits a sol–gel phase transition with a lower critical solution temperature (LCST). The underlying LCST is around room temperature. Cells were seeded with a polymer solution below LCST. When the mixture solution was injected, the cells were trapped in the hydrogel matrix under body temperature (above LCST). Polysaccharides such as hyaluronic acid and alginates were also employed as three-dimensional templates for cells

Correspondence to: J. Ding (jdding1@fudan.edu.cn).
Contract grant sponsor: National Science Foundation of China; contract grant numbers: 20221402, 20374015, and 30271293, and the Key Project.
Contract grant sponosr: The Key Grant of Chinese Ministry of Education; contract grant number: 305004.
Contract grant sponosr: The Award Foundation for Young Teachers from Ministry of Education; contract grant number: 863 project, 2004AA215170.
Contract grant sponsor: Science and Technology Developing Foundation of Shanghai; contract grant number: 973 project.



Scheme 1 Schematic diagram of the synthesis of poly(ethylene glycol)-*co*-oligo(hydroxy acid) copolymers, macromers, and hydrogels and the degradation of the associated hydrogel network.

with cations, such as Ca^{2+}, as crosslinking reagents.[19] These previously mentioned hydrogels are physical hydrogels because the crosslinking is not accompanied by a chemical reaction. Physical hydrogels are relatively unstable, especially under the environment of a body medium, and the biodegradation rates are difficult to control well in vitro and in vivo or to adjust in a large time span.

To develop a novel drug carrier, Hubbell et al.[20] synthesized a chemically crosslinked biodegradable hydrogel made from PEG-containing macromers in the presence of a photoinitiator sensitive to UV or visible light. The hydrogel was formed by free-radical polymerization in a mild manner under a simulated physiological environment. Burdick and Anseth[21] tried to employ PEG hydrogels as photoencapsulation matrices in bone tissue engineering. However, photopolymerization cannot be carried out uniformly in a large or thick system, especially in many clinical applications in which the light penetration depth is quite limited and light distribution is inhomogeneous.

A redox initiator was thus tried in this study, and the associated hydrogel behaviors were examined in detail. As model injectable materials, biodegradable hydrogels made from PEG-containing macromer solutions in phosphate-buffered saline (PBS) solutions were crosslinked by a water-soluble and room-temperature redox initiator system, as indicated in Scheme 1.

Because this is an early article for this kind of injectable hydrogel for tissue engineering, just the synthesis of the macromers and some physicochemical characterizations of the resulting hydrogels are reported to preliminarily confirm their potential feasibility. Some basic properties such as the gelation time, gel content, and biodegradable rate under a simulated physiological environment (PBS solution) have been investigated.

EXPERIMENTAL

Materials

PEGs denoted as PEG6K, PEG8K, and PEG10K were purchased from China Medicine (Group) Shanghai Chemical Reagent Corp. (Shanghai, China) and were purified by azeotropic distillation with benzene. Pluronic F127 (PEO_{99}–PPO_{65}–PEO_{99}) was purchased from Sigma (Milwaukee, WI). Stannous octoate and ε-caprolactone were obtained from Aldrich (Milwaukee, WI). D,L-Lactide was from Purac and was recrystallized from ethyl acetate. ε-Caprolactone was purified by reduced distillation. Ammonium persulfate (APS) was obtained from Aijian Chemical Co. (Shanghai, China) and recrystallized from distilled water. *N,N,N′,N′*-Tetramethylethylene diamine (TEMED; 99%) was purchased from Merck (Whitehouse Station, NJ). A PBS solution (pH 7.4) was used. All other chemicals were used without further purification.

Synthesis of acryloyl chloride

Acryloyl chloride was prepared by the reaction between benzoyl chloride and acrylic acid.[22] Briefly, a mixture of 72 g of acrylic acid, 281 g of benzoyl chloride, and 0.5 g of hydroquinone was distilled. The distilled component was collected in a receiver containing 0.5 g of hydroquinone at a temperature from 60 to 90°C and then distilled again. The distilled component was collected again at a temperature from 72 to 76°C.

Synthesis and characterization of the PEG/oligo(hydroxy acid) macromers

Ampule tubes were treated with a 10% (v/v) solution of trimethylchlorosilane in toluene, washed with acetone, and dried. PEG/oligo(hydroxy acid) block copolymers were synthesized by the ring-opening polymerization of cyclic monomers of hydroxy acid in the presence of PEG with stannous octoate as a catalyst. PEG8K (20 g) and 2.88 g of D,L-lactide were added to a dried 80-mL ampule tube connected to a vacuum joint, and the reaction mixture was preheated to the melting state to be mixed thoroughly. Subsequently, the mixture was cooled to room temperature. The catalyst solution (1.0 mL; 12.4 mg/mL of stannous octoate in toluene) was added to the tube. Then, the ampule tube was degassed in vacuo at 60°C for 4 h and filled with argon. Next, the tube was sealed off and placed in an oil bath at 130°C to mix the reactant, and then the temperature was elevated to 150°C for 24 h. After it cooled to room temperature, the ampule tube was crushed. The resultant copolymer was dissolved in dichloromethane, precipitated in anhydrous ether, filtered, washed several times with anhydrous ether, and dried in vacuo at room temperature (yield = 97%). Other copolymers were synthesized by the variation of the feed conditions of the cyclic monomers and PEG.

The degree of polymerization (DP) of the polyester was determined by 500-MHz ^{1}H-NMR spectra (DMX500 spectrometer, Bruker, Bremen, Germany). The samples in $CDCl_3$ were detected, and tetramethylsilane (TMS) was used as the internal standard. The block length of the oligoester (L) was calculated with the following equations:

$$L_{PCL} = L_{PEG} \times \frac{4(\text{Peak intensity of } CH_2 \text{ group of PCL})}{2(\text{Peak intensity of } CH_2 \text{ group of PEG})}/2$$

$$L_{PLA} = L_{PEG} \times \frac{4(\text{Peak intensity of } CH_3 \text{ group of PLA})}{3(\text{Peak intensity of } CH_2 \text{ group of PEG})}/2$$

Here L_{PEG} denotes DP of PEG. Because the copolymer has one oligo(hydroxy acid) block at each end of the PEG chain, the calculated value should be divided by 2.

Dichloromethane and triethylamine were dried over 3-Å molecular sieves. The dried PEG/oligolactide copolymer (18 g) was dissolved in 150 mL of dichloromethane in a 250-mL, round-bottom flask. Triethylamine (1.64 mL) was added to the flask. After the flask was cooled to 0°C in an ice bath, the mixture solution, composed of 1.47 mL of acryloyl chloride and 10 mL of dichloromethane, was added dropwise. The reaction mixture was stirred for 12 h at 0°C and for 12 h at room temperature under argon. Finally, the insoluble triethylamine salts were filtered. The filtrate was precipitated in excessive anhydrous ether, filtered, washed several times with anhydrous ether, and dried in vacuo at room temperature (yield = 92%). This PEG macromer is called PEG8K-LA_8-DA, which denotes a diacrylate-terminated macromer with the central PEG8K segment extended with eight lactoyl repeats per hydroxyl end of PEG according to the feed ratio. Other macromers were synthesized through the copolymerization of PEG and different cyclic monomers.

The chemical structures of the PEG/oligo(hydroxy acid) macromers were confirmed by Fourier transform infrared (FTIR) spectroscopy (Magna 550, Nicolet, Madison, WI). For FTIR analysis, KBr tablets were prepared by the dissolution of the samples in dichloromethane and evaporation of the solvent under light.

Preparation of the hydrogels and detection of the gelation time

The PEG macromer (0.5 g) was dissolved in 2.0 mL of a PBS solution. APS (3% w/w) and 1.0 μL/g TEMED were added to the macromer solution under agitation. Hydrogels were formed by the placement of the macromer mixture solutions at 37 ± 0.5°C in a thermostatic water bath.

The test-tube inverting method was employed to determine the gelation time.[23] Briefly, 25 wt % PEG macromer solutions with different initiator concentrations were sealed into a glass tube, which was 100 mm long and 8 mm in its inner diameter. The tube was immersed in the thermostatic water bath controlled at 37 ± 0.5°C. After some reaction time, the solution was monitored by the inversion of the glass tube. When the solution did not flow down in 1 min, this was regarded as the occurrence of gelation, and the period from the tube immersing into the bath to the gelation was defined as the gelation time.

Temperature change in polymerization

A PEG macromer solution (1.5 mL; 25 wt % in deionized water and with 4% APS by weight) was placed in

a glass tube covered with a lid. The tube was put in a thermostatic water bath at 37 ± 0.5°C, and a thermometer was put into the macromer solution preheated to 37°C. TEMED was then added to the solution. The temperature changes were recorded during the gelation process.

Moduli of the hydrogels

Pluronic F127 (30 wt %) and 25 wt % PEG macromers were prepared by the dissolution of the polymers in PBS. APS (5 wt %) and 3.0 μL/g TEMED were added to the macromer solution. The storage moduli of these two samples were monitored by dynamic oscillatory shear at 37°C with a rheometer (RheoStress RS75, Haake, Bersdorff, Germany). The polymer solutions were placed in a stainless steel mold with a parallel-plate geometry (60 mm in diameter). The mold was covered with a steel lid, and some deionized water was dropped around the mold to keep the moisture in the experiment. The storage modulus or elastic modulus was detected until 2.5 h after gelation. The oscillatory frequency was set to 1 Hz.

Gel content, water uptake, and swelling ratio

The obtained hydrogels were dried in vacuo with P_2O_5 at room temperature to a constant weight and weighed (W_1). The hydrogels were then extracted with chloroform in the Soxhlet extractor for 1 day to remove the unreacted macromers in the hydrogels. After being dried again in vacuo to a constant weight, the sample was weighed (W_2). The gel content (g) was expressed as follows:

$$g = \frac{W_2}{W_1} \times 100\% \quad (1)$$

Subsequently, the dried hydrogels were immersed in a PBS solution for 2 days to achieve swelling equilibrium. After being swabbed lightly with filter paper to remove the excess water on the surface of the hydrogels, the sample was weighed (W_3). The water uptake of the gel network was calculated as follows:

$$\frac{W_3 - W_2}{W_2} \quad (2)$$

The swelling ratio (R) in weight was determined as follows:

$$R = \frac{W_3}{W_2} \quad (3)$$

In vitro degradation

About 30 mg (W_0) of the dried hydrogels extracted by chloroform was reimmersed in 10 mL of PBS at 37 ± 0.5°C. After predetermined time intervals, the samples were taken out of PBS and dried in vacuo to a constant weight (W). The experiments were carried out in triplicate. The in vitro degradation of the hydrogels was examined gravimetrically as follows:

$$\frac{W_0 - W}{W_0} \times 100\% \quad (4)$$

RESULTS AND DISCUSSION

Synthesis of the PEG/oligo(hydroxy acid) macromers

The PEG/oligo(hydroxy acid) copolymers were synthesized by the ring-opening polymerization of cyclic monomers such as lactide and ε-caprolactone in the presence of PEG with stannous octoate as a transesterification catalyst. The reaction route of the copolymerization of PEG and the cyclic monomer is shown in Scheme 1. The hydroxyl groups at the two ends of a PEG chain initiated the ring-opening copolymerization of the cyclic monomer through the acyl–oxygen cleavage. The propagation reaction was completed by the stepwise addition of cyclic monomers to the hydroxyl groups of PEG chains.[24] Figure 1 shows the typical ^{1}H-NMR spectra of PEG/oligolactide and PEG/oligocaprolactone block copolymers. The CH_2 (~1.6 ppm) group belongs to oligocaprolactone, and CH_2 (~3.6 ppm) is from the PEG block. In the spectrum of the PEG/oligolactide block copolymer, CH_3 (~1.5 ppm) and CH (~5.2 ppm) belong to the oligolactide, whereas CH_2 (~3.6 ppm) is again from the PEG block. The DP of the polyester depends on the ratio of the cyclic monomer concentration to the PEG concentration. Some synthesized PEG/oligo(hydroxy acid) copolymers are shown in Table I. The DPs of the oligoesters were calculated from integral ^{1}H-NMR intensities. These results indicated that the PEG/oligo(hydroxy acid)s were produced as designed.

FTIR measurements of PEG, typical copolymers of PEG and oligo(hydroxy acid), and the corresponding macromers are shown in Figure 2. The FTIR spectra of PEG [Fig. 2(a)], its copolymer with oligolactide [Fig. 2(b)], and that with oligo(ε-caprolactone) [Fig. 2(c)] presented an absorption band at 3500 cm^{-1} due to the terminal hydroxyl group. This band disappeared for the PEG/oligo(hydroxy acid) macromers because of acrylation. Alternatively, a new and strong carbonyl band was seen at 1750 cm^{-1} in the spectra of PEG6K-LA_4 [Fig. 2(b)], PEG6K-CL_4 [Fig. 2(c)], and associated macromers [Fig. 2(d,e)], which confirmed the formation of block copolymers of PEG and oligo(hydroxy acid).

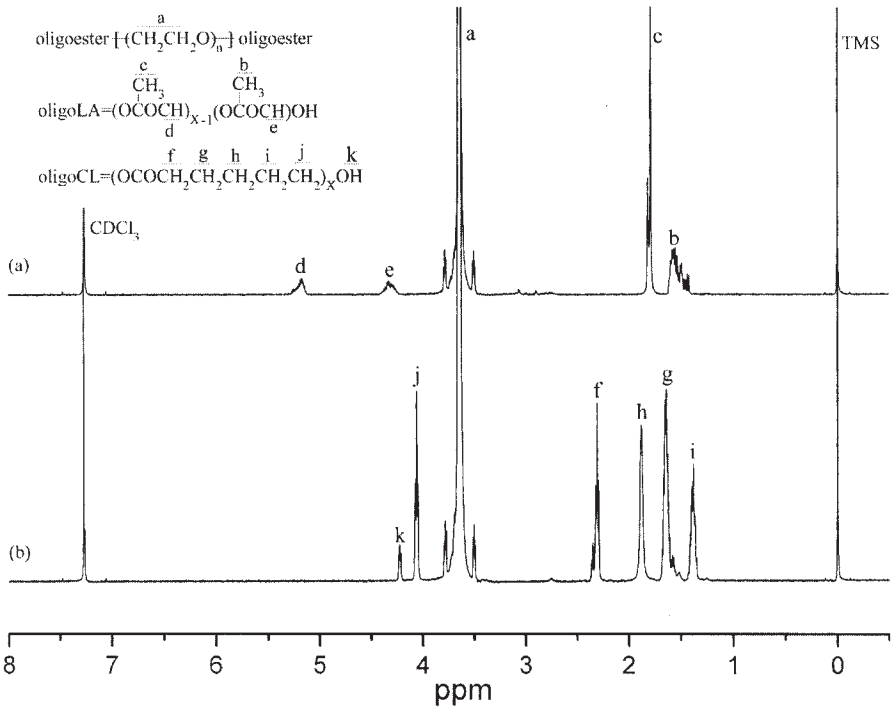


Figure 1 ^{1}H-NMR spectrum of the PEG copolymer with (a) oligolactide and (b) oligo(ε-caprolactone).

Preparation of the hydrogels and adjustment of the gelation time

The terminal hydroxyl groups in the PEG/oligo(hydroxy acid) block copolymer were subsequently converted to acryloyl groups by a reaction with acryloyl chloride. The number of acrylic groups in the PEG/oligo(hydroxy acid) block copolymer was expected to be two because one PEG molecule has two hydroxyl groups. Thus, the macromer aqueous solution could form a crosslinked, three-dimensional network in free-radical polymerization. In our experiments, the macromers were dissolved in PBS, and a redox initiation system was used, including APS as an initiator and TEMED as an accelerator. The reaction was carried out at 37°C. The crosslinking reaction scheme is also shown in Scheme 1.

The gelation time was subsequently investigated. Figure 3 shows the effect of the initiator concentration on the gelation time. A higher initiator concentration resulted in a shorter gelation time. At the same weight

TABLE I
Synthesis and Composition of the Macromers

Sample	Feed molar ratio [PEG/lactide or PEG/(caprolactone/2)]	DP of each oligoester block (determined by ^{1}H-NMR)	Macromer yield (%)
PEG6K-LA_4-DA	1:4	3.8	82
PEG6K-LA_8-DA	1:8	7.8	83
PEG6K-CL_4-DA	1:4	3.7	81
PEG8K-LA_2-DA	1:2	1.9	80
PEG8K-LA_4-DA	1:4	3.6	85
PEG8K-CL_4-DA	1:4	3.5	78
PEG10K-LA_2-DA	1:2	1.8	85
PEG10K-LA_4-DA	1:4	3.5	83

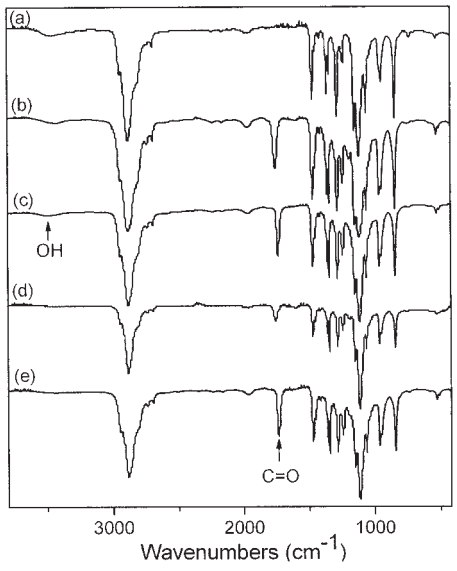


Figure 2 FTIR spectra of (a) PEG6K, (b) PEG6K-LA_4 copolymer, (c) PEG6K-CL_4 copolymer, (d) PEG6K-LA_4-DA macromer, and (e) PEG6K-CL_4-DA macromer.

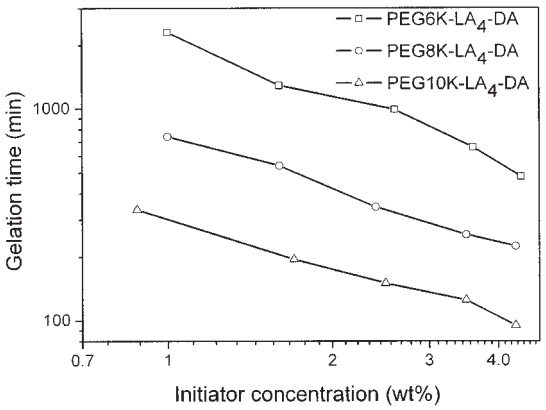


Figure 3 Dependence of the gelation time on the initiator concentration for different PEG-containing macromers with a macromer concentration of 25 wt % in PBS at 37°C. The concentration of TEMED was 1.0 μL/g in the macromer solutions.

concentration of the macromer, longer polymer chains led to shorter gelation times, and this is presumably attributable to the higher molar ratio between the initiator and the macromers. Macromer aqueous solutions are expected to be used under physiological circumstances, so a water-soluble radical initiation system (APS/TEMED) was employed in our work. Here, TEMED plays the role of an accelerator to catalyze the crosslinking reaction.[25]

The gelation time should not be very long. Otherwise, if injected into the body, the macromer solutions will presumably diffuse to the surrounding tissue ahead of hydrogel formation. Therefore, it is necessary to use TEMED with APS for the accomplishment of the crosslinking reaction. The gelation time as a function of the concentration of the accelerator in macromer solutions is shown in Figure 4. With an increasing concentration of the accelerator, gelation occurred quickly. When the concentration of TEMED was increased from 0.6 to 1.3 μL/g in a macromer solution, the gelation time in the case of PEG8K-LA_2-DA was reduced from 450 to 15 min. In contrast, it took 43 h to form a gel without TEMED, and this could not be accepted for a feasible injectable biomaterial.

On the other hand, the gelation should not be very short either. Otherwise, the surgeons may not have sufficient time to perform the injection. Fortunately, the gelation time was highly adjustable in our experiments (Figs. 3 and 4). In our opinion, the appropriate gelation time might be around 10 min, which is available in our hydrogel systems.

Temperature change during polymerization

In this work, a water-soluble initiator system including APS and TEMED was used to trigger polymerization. As a potential injectable tissue engineering biomaterial, the macromer solution might form a gel *in situ* to trap cells after the mixture of the macromer aqueous solution and cells is injected into the body. A radical polymerization is generally an exothermic reaction. Thus, the encapsulated cells might be injured if a great amount of heat is released during the process of polymerization. The striking polymerization heat might lead to the necrosis of surrounding tissues and thus failure of implantation.[26] Figure 5 shows the temperature change of macromer solutions in PBS when gelation occurred in a thermostatic water bath at 37°C. The crosslinking reaction resulted in a slight temperature increase up to about 1.1°C. Therefore, within a reasonable gelation time range (tens of minutes), hydrogel formation might not lead to significant harm to embedded cells and surrounding tissues as far as the exothermic effect is concerned. Biological experiments will be performed to confirm this viewpoint in the future.

Gel content and water uptake

The gel content is a basic parameter to calibrate gel formation due to polymerization or crosslinking because not all the macromers eventually join the gel

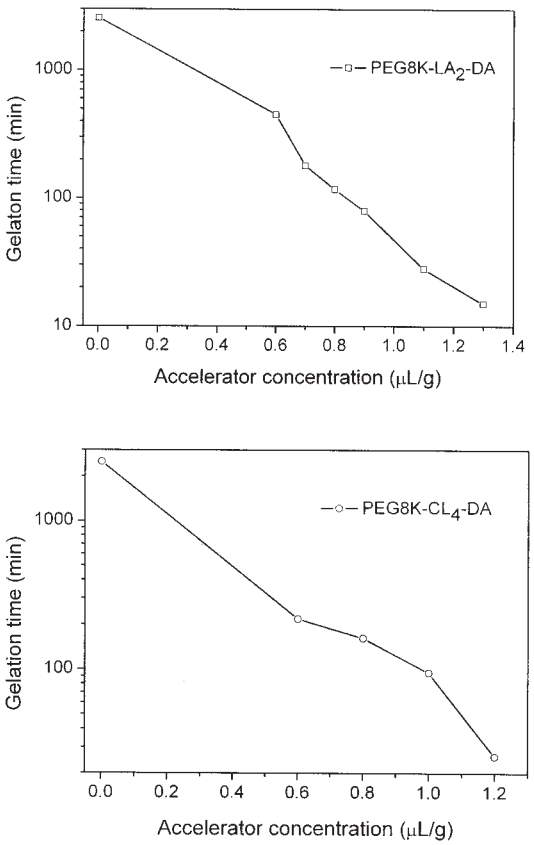


Figure 4 Effect of the accelerator concentration on the gelation time for the marked macromers in PBS at 37°C. The concentration of APS was 1 wt %.

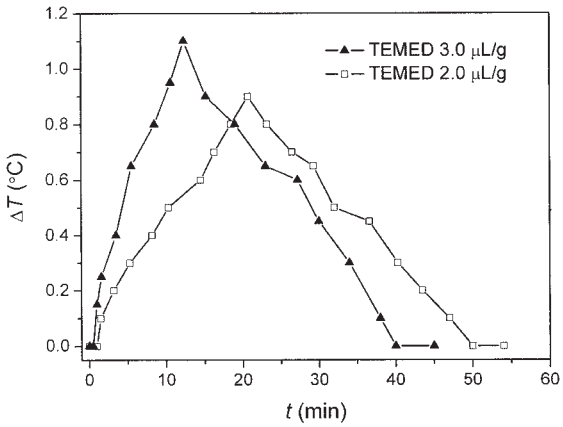


Figure 5 Temperature change (ΔT) during the chemical crosslinking reaction of macromers (PEG10K-LA_4-DA) with a concentration of 25 wt % in PBS solutions at the marked accelerator concentrations in a water bath at 37°C. The APS concentration was 4.0 wt %.

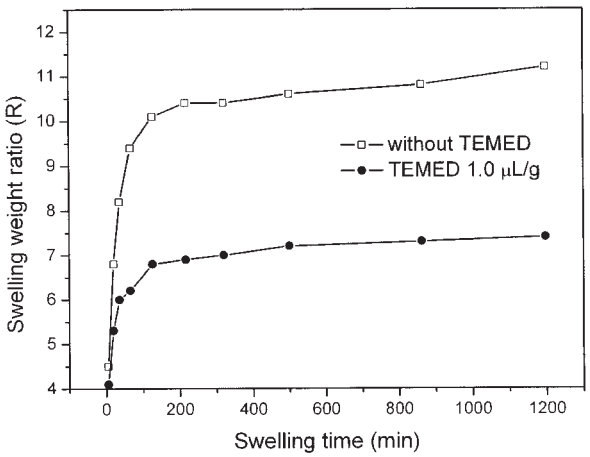


Figure 6 Swelling ratio versus the swelling time after dried gels were placed in deionized water. The hydrogels were prepared through the crosslinking of 25 wt % PEG10K-LA_2-DA at 37°C with 3 wt % APS as an initiator but with 1.0 μL/g TEMED or without TEMED as an accelerator.

network. Table II shows the gel content and water uptake prepared from a 25 wt % PEG8K-LA_2-DA macromer solution in PBS. In the examined range of TEMED concentrations, the resulting gel content and water uptake did not exhibit much difference. The gel content was a little bit increased, but still more than 30% of the macromers did not join the network. The gel content is required to increase in the future.

We presumed that a mixture solution of macromers and cells should form a gel under physiological conditions after being injected into the body and that cells would thus be entrapped in the hydrogel matrix. Hydrogels could absorb the body fluid and be swollen. The swollen hydrogels might contact tightly with the surrounding tissue and so fill the damaged tissue.

If the initial macromer concentration and the gel content in the resulting hydrogels are denoted as c and g, the weight percentage of the polymeric network in the hydrogel (a mixture of the network and water, but free of the remaining macromers) after polymerization could be determined as follows:

TABLE II
Gel Content and Water Uptake of Hydrogels Made from PEG8K-LA_2-DA (25 wt %) with Different Accelerator Concentrations in PBS at 37°C

Accelerator concentration (μL/g)	Gel content (%)	Water uptake
0.6	66.8	10.5
0.7	66.6	10.9
0.8	65.8	9.2
0.9	67.8	9.8
1.1	69.2	10.8
1.3	72.1	9.9

The concentration of APS was 1 wt %.

$$cg/(cg + 1 - c) \quad (5)$$

When g was presumed to be 66.7% at c = 25%, the weight percentage of the skeletal network in the hydrogel was about 18%, and the water concentration in the hydrogels was thus about 82% after polymerization. In fact, the water concentration was about 90% when the extracted gel was swollen to equilibrium in PBS after 2 days (Table II). Therefore, the hydrogels might not lead to strong pressure to the surrounding tissues because of the absorption of the surrounding body medium after gel formation.

The swelling kinetics of a dried gel in water are presented in Figure 6. In the early stage of immersion in deionized water, the dried hydrogel was quickly swollen because of very fast water absorption. Within the first 1 h, the swelling ratios reached 6.2 for the hydrogels prepared with TEMED as an accelerator and 9.4 for the hydrogel prepared without TEMED. After about 24 h, the hydrogels exhibited the state of the swelling equilibrium, and the swelling ratios increased to 7.5 and 11.3, respectively. The swelling ratio of the hydrogels prepared with TEMED as an accelerator was always smaller than that of the hydrogel prepared without TEMED, and this may be attributed to the increasing crosslinking density due to TEMED as an accelerator.

Moduli of the chemical gel and physical gel

As we know, biomaterials should be of a certain mechanical stiffness for various applications in medical fields.[14,27] In this work, we employed a parallel-plate rheometer to detect the storage modulus of hydrogels in a dynamic shear mode subject to oscillatory shear

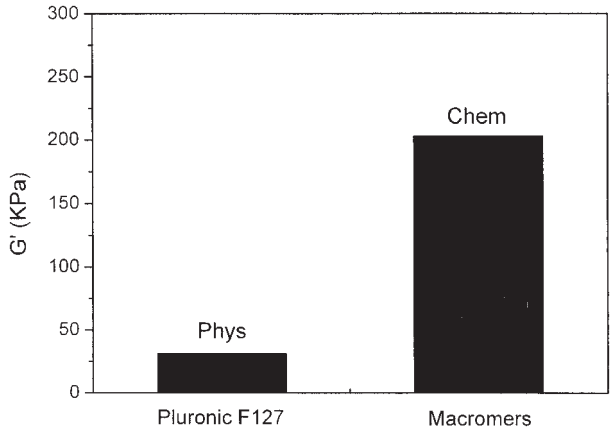


Figure 7 Elastic modulus (G') of a physically crosslinked hydrogel made from 30 wt % Pluronic F127 and of a chemically crosslinked hydrogel made from a 25 wt % PEG6K-LA_8-DA macromer solution.

flow. The macromer solutions were in situ crosslinked between the two plates. For comparison, we also detected a physically crosslinked hydrogel composed of 30 wt % Pluronic F127. The mixture presented a sol–gel phase transition with an increase in temperature and was in the gel state at 37°C. This hydrogel with the same concentration has been employed as an injectable cell scaffold in tissue engineering.[6] The rheological measurements at a low shear frequency are presented in Figure 7. The storage modulus of the chemical hydrogel was 0.203 MPa, whereas that of the physical hydrogel was 0.031 MPa. The aggregate modulus of natural cartilage is about 0.9 MPa.[27] The chemically crosslinked hydrogel is reasonably stronger than the Pluronic physical hydrogel and closer to the cartilage as far as the modulus is concerned.

In vitro degradation of the hydrogel

Biodegradability is very important for many implanted biomaterials.[12,14,15,27,28] The hydrogels with three-dimensional, crosslinked networks were subjected to the hydrolysis of the oligoester extending the central PEG. As shown in Scheme 1, hydrolysis was caused at each end of the crosslinked polymer chain, and the degradation products were presumably PEG, hydroxy acid, and oligomeric acrylic acid.[20] Figure 8 shows the degradation behaviors of the hydrogels with different lengths and types of oligoesters. It is reasonable that hydrogels made from the shorter oligoester blocks presented relatively slow degradation because of the smaller probability of hydrolysis of oligoester blocks. The degradation of hydrogels containing oligo(ε-caprolactone) was slower than that of those containing oligolactide, and this was attributed to the more hydrophobic nature of oligo(ε-caprolactone) versus that of oligolactide. Hydrogels made from the macromer of PEG8K-CL_4-DA took 40 days to degrade 97.6% [Fig. 8(a)], whereas those made from the macromer of PEG6K-LA_4-DA degraded almost completely within 33 days [Fig. 8(b)]. Therefore, the alteration of the oligoester composition is more effective for tailoring the degradation rate of hydrogels.

Figure 9 shows the degradation behavior of hydrogels prepared with different accelerator concentrations. It took about 26 days to degrade from 75 to 82% for those hydrogels. Hydrogels made with higher TEMED concentrations presented relatively slow degradation because of the increase in the crosslinking degree of the network, and this was consistent with the aforementioned swelling behavior in our experimental observations. Therefore, our experiments demonstrate that the degradation rate of a hydrogel made from PEG-containing macromers can be adjusted by

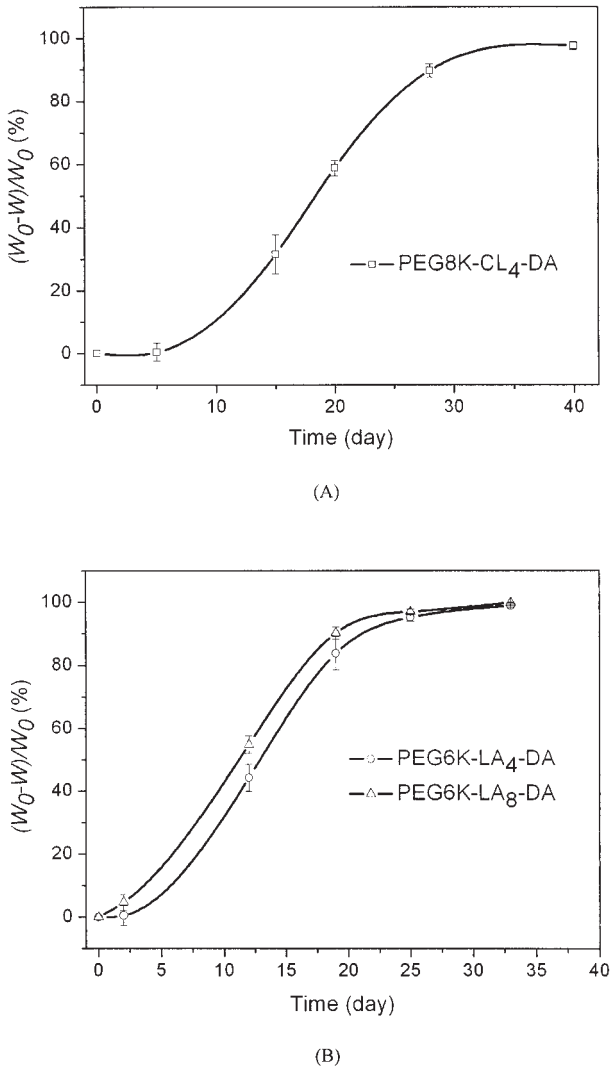


Figure 8 *In vitro* degradation of a hydrogel through the crosslinking of (A) PEG8K-CL_4-DA and (B) PEG6K-LA_4-DA or PEG6K-LA_8-DA in PBS at 37°C. The concentration of the macromer solutions was 25 wt %, and that of APS was 2 wt %.

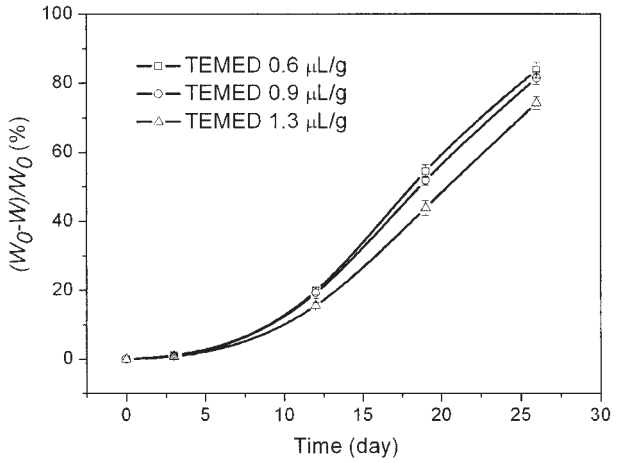


Figure 9 In vitro degradation of a hydrogel through the crosslinking of PEG8K-LA_2-DA with different accelerator concentrations in PBS at 37°C. The concentration of the macromer solution was 25 wt %, and that of APS was 2 wt %.

the alteration of the crosslinking degree, the hydrolytic susceptibility of the oligoester group, and so forth.

CONCLUSIONS

In this work, hydrogels were prepared from PEG-containing macromer solutions in PBS at 37°C with a redox initiation system with APS as an initiator and TEMED as an accelerator. The macromers had a hydrophilic central part of PEG extended with oligo(hydroxy acid) and terminated with acryloyl groups. PEG/oligo(hydroxy acid) copolymers were synthesized by the ring-opening polymerization of lactide or ε-caprolactone in the presence of terminal hydroxyl groups of PEG with stannous octoate as a catalyst. FTIR and ^{1}H-NMR were carried out to confirm the structures of the PEG/oligo(hydroxy acid) block copolymers and macromers. The gelation time decreased with an increasing concentration of APS or TEMED, and a reasonable gelation time region could be obtained. The temperature change of the hydrogels during the process of the chemical crosslinking reaction was not significant at a reasonable gelation rate. The modulus of the chemically crosslinked hydrogel was higher than that of the associated physically crosslinked hydrogel. Furthermore, the degradation rates of the hydrogels under simulated physiological conditions were tunable by the length and composition of the oligoester blocks and also by the crosslinking degree. Therefore, it is possible to tailor the degradation rate to a larger extent to meet diverse requirements for various applications in medical fields. On the basis of these results, mixture solutions of macromers and cells might be injected into the body and lead to in situ hydrogel formation. The chemical hydrogel is assumed to be a potential cell scaffold for tissue engineering.

References

1. Hoffman, A. S. Adv Drug Delivery Rev 2002, 54, 3.
2. Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. Eur J Pharm Biopharm 2000, 50, 27.
3. Horak, D.; Dvorak, P.; Hampl, A.; Slouf, M. J Appl Polym Sci 2003, 87, 425.
4. Peppas, N. A.; Keys, K. B.; Torres-Lugo, M.; Lowman, A. M. J Controlled Release 1999, 62, 81.
5. Harris, J. M. Polyethylene Glycol Chemistry, Biotechnical and Biomedical Applications; Plenum: New York, 1992.
6. Cao, Y. L.; Rodriguez, A.; Vacanti, M.; Ibarra, C.; Arevalo, C.; Vacanti, C. A. J Biomater Sci Polym Ed 1998, 9, 475.
7. Rathna, G. V. N. J Appl Polym Sci 2004, 91, 1059.
8. Ward, J. H.; Peppas, N. A. J Controlled Release 2001, 71, 183.
9. Leach, J. B.; Schmidt, C. E. Biomaterials 2005, 26, 125.
10. Lu, S. X.; Anseth, K. S. Macromolecules 2000, 33, 2509.
11. Han, D. K.; Hubbell, J. A. Macromolecules 1997, 30, 6077.
12. Bryant, S. J.; Bender, R. J.; Durand, K. L.; Anseth, K. S. Biotechnol Bioeng 2004, 86, 747.
13. Langer, R.; Vacanti, J. P. Science 1993, 260, 920.
14. Wu, L. B.; Ding, J. D. Biomaterials 2004, 25, 5821.
15. Lu, L.; Peter, S. J.; Lyman, M. D.; Lai, H. L.; Leite, S. M.; Tamada, J. A.; Uyama, S.; Vacanti, J. P.; Langer, R.; Mikos, A. G. Biomaterials 2000, 21, 1837.
16. Chen, G. P.; Ushida, T.; Tateishi, T. Biomaterials 2001, 22, 2563.
17. You, Y.; Min, B. M.; Lee, S. J.; Lee, T. S.; Park, W. H. J Appl Polym Sci 2005, 95, 193.
18. Khang, G.; Choee, J. H.; Rhee, J. M.; Lee, H. B. J Appl Polym Sci 2002, 85, 1253.
19. Hubbell, J. A. U.S. Pat. 6,129,761 (2000).
20. Sawhney, A. S.; Pathak, C. P.; Hubbell, J. A. Macromolecules 1993, 26, 581.
21. Burdick, J. A.; Anseth, K. S. Biomaterials 2002, 23, 4315.
22. Stempel, G. H.; Cross, R. P.; Mariella, R. P. J Am Chem Soc 1950, 72, 2299.
23. Leong, B. M.; Lee, D. S.; Shon, J. I.; Bae, Y. H.; Kim, S. W. J Polym Sci Part A: Polym Chem 1999, 37, 751.
24. Kricheldorf, H. R.; Kreiser-Saunders, I.; Stricker, A. Macromolecules 2000, 33, 702.
25. Feng, X. D.; Guo, X. Q.; Qiu, K. Y. Makromol Chem 1988, 189, 77.
26. Temenoff, J. S.; Mikos, A. G. Biomaterials 2000, 21, 2405.
27. Freed, L. E.; Langer, R.; Martin, I.; Pellis, N. R.; Vunjak Novakovic, G. Proc Natl Acad Sci USA 1997, 94, 13885.
28. Anderson, J. M.; Hiltner, A.; Wiggins, M. J.; Schubert, M. A.; Collier, T. O.; Kao, W. J.; Mathur, A. B. Polym Int 1998, 46, 163.