Synthesis and Characterization of Photo-Cross-Linked Networks Based on L-Lactide/Serine Copolymers†

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ABSTRACT: New lactide-based polydepsipeptide polymer networks and cross-linked beads have been prepared by UV photopolymerization of acrylated poly(L-lactic acid-*co*-glycolic acid-*co*-L-serine) [PLA-(Glc-Ser)]. These materials have been developed for use as polymer scaffolds in tissue engineering, cell encapsulation, and injectable drug delivery, which have ligand-immobilizable and biodegradable characteristics. The acrylated PLA(Glc-Ser) cross-linked polymer networks obtained were glassy and transparent, and the gel content was approximately 90%. The networks showed relatively low swelling in water, due to their cross-linked nature, but are easily swelling in chloroform and in dimethyl sulfoxide. The acrylate polymers on copolymerization with 2-hydroxyethyl methacrylate (HEMA) resulted in cross-linked networks [PLA(Glc-Ser)/HEMA], which were swelling in water and in DMSO showing the potential of the polymer in hydrogel applications. The modified PLA(Glc-Ser) beads prepared by UV-initiated suspension polymerization were characterized by optical microscopy and FTIR. These degradable networks and beads should be useful as polymer scaffolds for biomaterial applications.

Introduction

Poly(α-hydroxy esters) such as poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) are biodegradable polymers which have been used in medicine as sutures and in tissue engineering as polymer scaffolds.[1−3] PLA and their copolymers ultimately degrade to chemicals naturally found in vivo. These materials are relatively biocompatible and appropriately biodegradable but lack the ability to interact biospecifically with cells.[4] To overcome this, biodegradable polydepsipeptides, alternating copolymers of α-amino acids and α-hydroxy acids, with functional side groups may be useful.[5−12] Recently, it was reported that appropriately modified polydepsipeptides could find applications in biomaterial use as polymer scaffold and biocompatible gel preparation.[14,18]

Hydrogels have been a recent focus for the encapsulation of cells in tissue engineering.[13−15] Hydrogels are insoluble polymer networks that provide a physical barrier to retain cells in conjunction with a high water content allowing for diffusion of nutrients and waste. Hubbell and co-workers have synthesized acrylates based on lactic acid polymers and photopolymerized the acrylate functionalities in the presence of cells to encapsulate the cells in the resulting gel without damaging them.[16] They have also utilized photopolymerization for the synthesis of biocompatible hydrogels based on poly(ethylene oxide).[17] In an another study, Elisseeff et al. reported the UV polymerization of lactic acid/aspartic acid polymers by the methacrylate grafting method.[18] Photopolymerization with these polymers produces minimal heat and requires small quantities of photoinitiator, resulting in a biocompatible gel system that may be polymerized in vivo.

The present work combines the synthesis of degradable polydepsipeptides and hydrogel photopolymerization technology to form polymer networks and cross-linked beads based on acrylated PLA(Glc-Ser) and their subsequent characterization. We have reacted the serine units of PLA(Glc-Ser) with acryloyl chloride to produce acrylate functional groups along the polymer backbone. Photopolymerization of the acrylated polymers should yield a cross-linked gel in bulk polymerization and beads by suspension polymerization. The hydrophilicity of the acrylated PLA(Glc-Ser) copolymers can be increased by copolymerizing with 2-hydroxyethyl methacrylate (HEMA). The PLA(Glc-Ser)/HEMA copolymer matrix is swelling in DMSO and water, indicating the potential of the polymer in hydrogel applications and in injectable drug delivery. This is significant because cross-linked acrylate polymers and beads have attracted attention as carrier matrices in medical and biological applications, such as immobilization technologies, drug delivery systems, and cell culturing.[19]

Experimental Section

Materials. The *O*-benzyl-L-serine (Ser(Bzl), Kokusan Chemicals), stannous octanoate (Sn(II)Oct, Chameleon), 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), and 2,2-dimethoxy-2-phenylacetophenone (benzyl dimethyl ketal, BDMK) (Wako Chemicals) were used as received. Acryloyl chloride (Wako Chemicals) was distilled before use. L-Lactide (Aldrich) was recrystallized from dry toluene and dried over P_2O_5 before use. All other chemicals were reagent grade and were used without further purification.

Methods. The number- and weight-average molecular weights (M_n and M_w) of the resultant polymers were determined with a Tosoh 8000 series high-performance liquid chromatograph equipped with columns for gel permeation chromatography (GPC) packed with TSK Gel G 7000 HHR, G 6000 HHR, G 4000 HHR, and G 2000 HHR connected in this order at 35 °C and an RI 8020 detector, using a chromatocorder 21 data system. Tetrahydrofuran was used as eluent, and the average molecular weight of the polymers was calculated based on calibrations using polystyrene standards. ^{1}H NMR and ^{13}C

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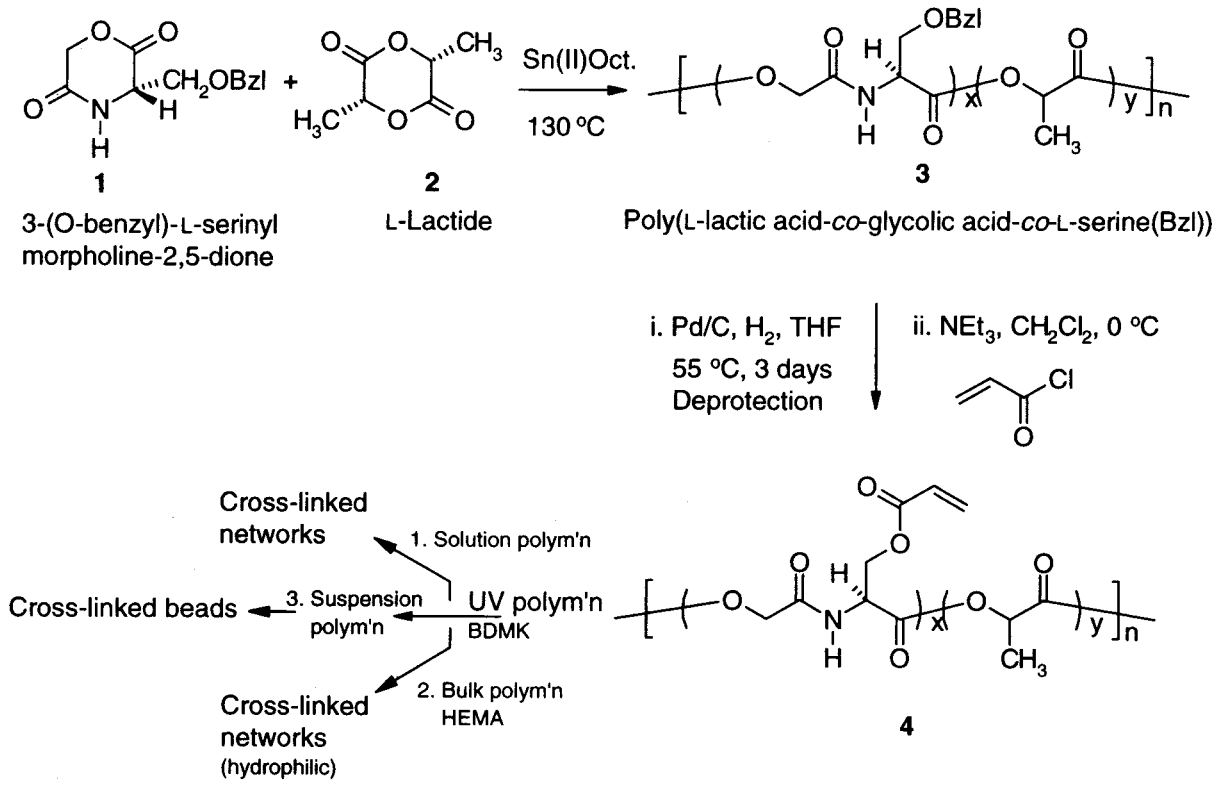
Table 1. ^{1}H NMR Chemical Shifts ($CDCl_3$) of Selected Copolymers of 3-(*O*-Benzyl)-L-serinylmorpholine-2,5-dione (1) with L-Lactide

comonomer	mol % **1** feed	mol % **1** calcd[a]	chemical shifts (ppm)
L-lactide	0.00	0.00	δ = 1.58 (d, J = 7.1 Hz, 3H, C**H**$_3$), 5.16 (q, J = 7.1 Hz, 1H, C**H**)
L-lactide	3.92	3.80	δ = 1.58 (d, J = 7.1 Hz, 3H, C**H**$_3$), 5.16 (q, J = 7.1 Hz, 1H, C**H**) plus: 3.6−4.2 (m, 2H, OC**H**$_2$), 4.45−4.53 (m, 1H, NHC**H**), 4.61 (s, 2H, OC**H**$_2$), 5.08 (m, 2H, C**H**$_2$Ph), 6.78 (d, N**H**), 7.34 (m, 5H, **Ph**)

[a] Calculated from ^{1}H NMR values.

Scheme 1. Synthesis of Lactic Acid Based Photo-Cross-Linkable Polymers



NMR spectroscopy was performed with a JEOL Alpha 500 NMR spectrometer using $CDCl_3$ as solvent and tetramethylsilane as an internal standard. FTIR spectra were recorded with a Perkin-Elmer instrument model 1600 on neat thin films on silicon wafers. Thermal transition data were collected with a MAC Science DSC-3100. The sample size ranged from 5 to 10 mg, and indium was used for the temperature calibration. Each sample was subjected to a heat−cool−heat cycle in air from −10 to 200 °C with a heating rate of 10 °C/min. The values are reported from the second heating cycle except for the cross-linked polymers which were only heated once.

Monomer Synthesis and Copolymerization. The monomer 3-(*O*-benzyl)-L-serinyl-morpholine-2,5-dione **(1)** was synthesized as per the method reported earlier.[12] Polymerization flasks (25 mL) were silanized using trimethylsilyl chloride (20 vol % in toluene), followed by repeated washings with toluene and methanol. The flasks were equipped with a stirring bar and dried at 120 °C overnight. Subsequently, the flasks were cooled to room temperature in a vacuum and refilled with dry argon. The monomers were added to the polymerization flasks in a drybox. A total of 10 mmol of the monomer was weighed into the flasks. The required amount (10−100 μL) of a freshly prepared 0.2 M solution of Sn(II)Oct in toluene was added using a glass syringe, to give the desired monomer initiator (M/I) mole ratio. The samples were put under high vacuum for 1 h to eliminate the toluene used to add the initiator. The flask was flushed five times with argon over this period. During the last 30 min of the vacuum cycle, the samples were gently heated with a heating mantle for few minutes, and the flask was sealed by turning the stopcock; the flasks were placed in an oil bath at 130 °C for the required time. The polymerization was stopped by removing the flasks from the oil bath and allowing them to cool to room temperature. Purification was performed by dissolving the polymer sample in CH_2Cl_2 and slowly adding into an excess of methanol. The sample size was generally 0.8−1.5 g. The polymers were collected and dried in vacuo at 40 °C for 4 h. The proton NMR analysis results of the selected copolymers are given in Table 1.

Deprotection of the Copolymers. *Benzyl deprotection*. The copolymers (0.5 g) were dissolved in 10 mL of tetrahydrofuran, and 125 mg of 10% palladium/carbon (Pd/C) was added. Hydrogen was bubbled through the suspensions with stirring for 3 days at 55 °C. The catalyst was removed by filtration over Celite 545. The filtrate was added dropwise to a 20-fold excess of diethyl ether/*n*-hexane, 2:1. The precipitated polymer was collected and dried in vacuo at 40 °C for 5 h. ^{1}H NMR ($CDCl_3$): δ = 1.58 (d, J = 7.1 Hz, 3H, C**H**$_3$), 3.6−4.2 (m, 2H, OC**H**$_2$), 4.45−4.53 (m, 1H, NHC**H**), 4.61 (s, 2H, OC**H**$_2$), 5.16 (q, J = 7.1 Hz, 1H, C**H**), 6.78 (d, 1H, N**H**).

Synthesis of Acrylate Derivatives. The deprotected polymers were further modified through the hydroxyl group of serine residue by reacting with acryloyl chloride. In a typical reaction, the PLA(Glc-Ser) copolymer was dissolved in anhydrous CH_2Cl_2, and the solution was placed in a three-necked flask equipped with magnetic stirrer and a dropping funnel. The contents of the flask were cooled to 0 °C, and triethylamine (NEt_3) was added. The solution was stirred, and then, freshly distilled acryloyl chloride in CH_2Cl_2 was added dropwise to the solution. The stirring was continued for 30 min at 0 ± 2 °C and then for 2 h at room temperature. The polymer was precipitated from cold methanol and further purified by repeated precipitation from CH_2Cl_2 to methanol. Yield: 85%. IR (neat, cm^{-1}): 3367 (ν NH), 2945 (ν CH), 1758 (ν C═O, ester),

Table 2. Ring-Opening Copolymerization of 3-(*O*-Benzyl)-L-serinylmorpholine-2,5-dione (1) and L-Lactide[a]

sample	mol % **1** feed	mol % **1** calcd[b]	yield (%)	M_n[c] × 10^{-4}	M_w[c] × 10^{-4}	T_g (°C)	T_m (°C)
1	0.00	0.00	85	3.6	9.2	59.9	172.5
2	0.50	0.43	79	3.2	6.8	58.3	170.7
3	1.83	1.69	75	3.0	5.7	56.5	167.5
4	3.92	3.80	70	2.9	5.4	54.8	163.8

[a] Temperature: 130 °C (48 h). Catalyst: Sn(II)Oct, mole ratio M/I of 1000. [b] Calculated from ^{1}H NMR values. [c] All the molecular weight data were obtained on protected copolymers.

1660 (ν C=O, amide), 1633.9 (double bond). ^{1}H NMR ($CDCl_3$): δ = 1.58 (d, J = 7.1 Hz, 3H, C**H**$_3$), 3.6−4.2 (m, 2H, OC**H**$_2$), 4.45−4.53 (m, 1H, NHC**H**), 4.61 (s, 2H, OC**H**$_2$), 5.16 (q, J = 7.1 Hz, 1H, C**H**), 5.8−6.4 (m, 3H, C**H**=C**H**$_2$), 6.78 (d, 1H, N**H**). ^{13}C NMR ($CDCl_3$): δ = 16.6 (**C**H$_3$), 45.75 (CH**C**H$_2$), 53.39 (O**C**H$_2$), 60.34 (**C**HNH), 68.97 (**C**HCH$_3$), 128.41, 131.36 (**C**H=**C**H$_2$), 168.85−169.55 (carbonyl carbons).

UV Polymerization of the Acrylated Copolymers. Scheme 1 represents the synthetic reaction of PLA(Glc-Ser) polymer networks. The cross-linked networks were prepared by different methods: (1) solution polymerization, (2) bulk polymerization, and (3) suspension polymerization. (1) A 25% w/v solution of 0.2 g of copolymer **4** and BDMK [1% w/w on the basis of PLA(Glc-Ser)] dissolved in CH_2Cl_2 was prepared. The mixture was coated on the bottom of the Petri dish using an applicator and irradiated by a 100 W medium-pressure mercury ultraviolet source at an intensity of 10 mW/cm^2 to produce lactic acid/serine polymer networks. The photopolymerization was continued until gelation occurred. (2) Approximately 10 mg of PLA(Glc-Ser) acrylated derivative **4** was placed in a tissue culture insert with a diameter of 0.9 cm. Ten microliters of initiator solution containing 300 mg of BDMK as initiator and ethylene glycol dimethacrylate (EGDMA) as a cross-linking agent in 1 mL of HEMA was mixed with the dry polymer in order to perform a bulk polymerization. The insert was then placed under an 100 W medium-pressure mercury ultraviolet source for 5 min or until the polymer appeared solid. Gels had a diameter of 0.9 cm and a height of 1−4 mm. (3) In a typical experiment, 50 mL of a 2 wt % gelatin solution was used as the suspension medium. Acrylated copolymer **4** (25 mg), EGDMA as a cross-linking agent, and BDMK [1% w/w on the basis of PLA(Glc-Ser)] as initiator were dissolved in 1,2-dichlorobenzene (0.5 mL), and the mixture was flushed with argon and add to the degassed suspension medium using a microsyringe, while stirring (at constant stirring rate of 500 rpm). The reaction was continued for 3 h irradiated by a 100 W medium-pressure mercury ultraviolet source to produce polymer beads. The polymer beads obtained were filtered, washed with water, and further dispersed in ethyl alcohol to remove the organic solvents used for dissolution. The dispersion was sonicated for 1 h, and the beads were collected and washed with distilled water again, then filtered, and dried in a vacuum oven at 50 °C for 24 h.

Swelling Studies. The polymer networks prepared by solution polymerization were dried under vacuum at 50 °C for 1 day, weighed (W_1), and then extracted with chloroform at room temperature for 1 day. The films were dried again and weighed (W_2). The gel content was calculated as (W_2/W_1) × 100%. Subsequently, the films were immersed in selected solvents at room temperature for 24 h and weighed (W_3). The solvent absorption was calculated as [($W_3 - W_2$)/W_3] × 100%. Solvent content of the PLA(Glc-Ser)/HEMA grafted copolymers by bulk polymerization was also studied. The cross-linked polymer was thoroughly washed with water and DMSO to remove any unreacted soluble fraction. The samples were vacuum-dried for 2 days, after which dry weights were obtained. The gels were reswollen for 24 h in water (phosphate-buffer, pH 6.8) or in DMSO. Excess solvent was removed by lightly patting the gels with a towel, and swollen weights were obtained. The swelling ratio (degree of swelling) was calculated as [($W_3 - W_2$)/W_2].

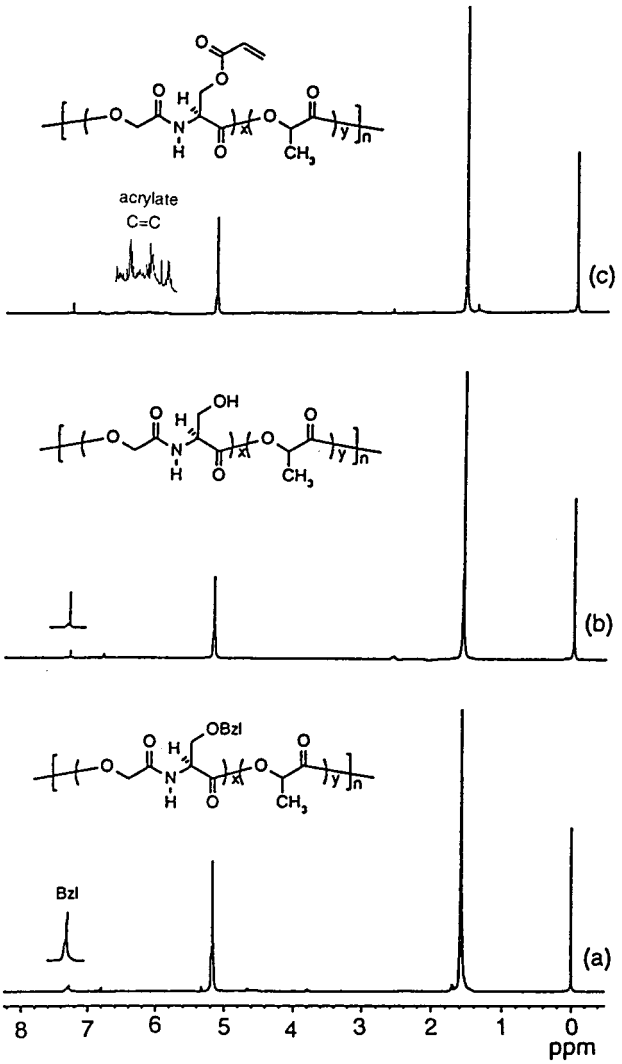


Figure 1. ^{1}H NMR ($CDCl_3$) of (a) protected, (b) deprotected, and (c) acrylated polymer.

Results and Discussion

Synthesis of Monomer 1 and Copolymerization with L-Lactide. The synthesis of 3-(*O*-benzyl)-L-serinylmorpholine-2,5-dione **(1),** which consists of *O*-benzyl-L-serine [Ser(Bzl)] and glycolic acid (Glc), was performed as reported in our earlier work.[12] The ring-opening copolymerization of **1** with L-lactide was performed using stannous octanoate as initiator with a M/I ratio of 1000, at 130 °C for 48 h as shown in Scheme 1. The bulk copolymerization of the morpholine-2,5-dione derivative **1** with L-lactide yielded copolymers containing serine with a molecular weight range of 1 × 10^4 as determined by GPC, given in Table 2. The glass transition temperature, T_g, and the melting temperature, T_m, of the samples (Table 2) decreased as the serine monomer content in the feed was increased. The presence of the serine residue at relatively low percentages disrupts the crystalline region slightly, causing the decrease in T_m. The benzyl protective groups of copolymers were removed by catalytic hydrogenation using Pd/C (10%) as catalyst. ^{1}H NMR analysis of the resulting copolymers demonstrated almost complete removal of the protecting groups by the absence of proton signals of the benzyl group at δ = 5.08 and 7.34 ($CDCl_3$) (Figure 1). The GPC data of the deprotected copolymers suggested that the main-chain cleavage of copolymers did

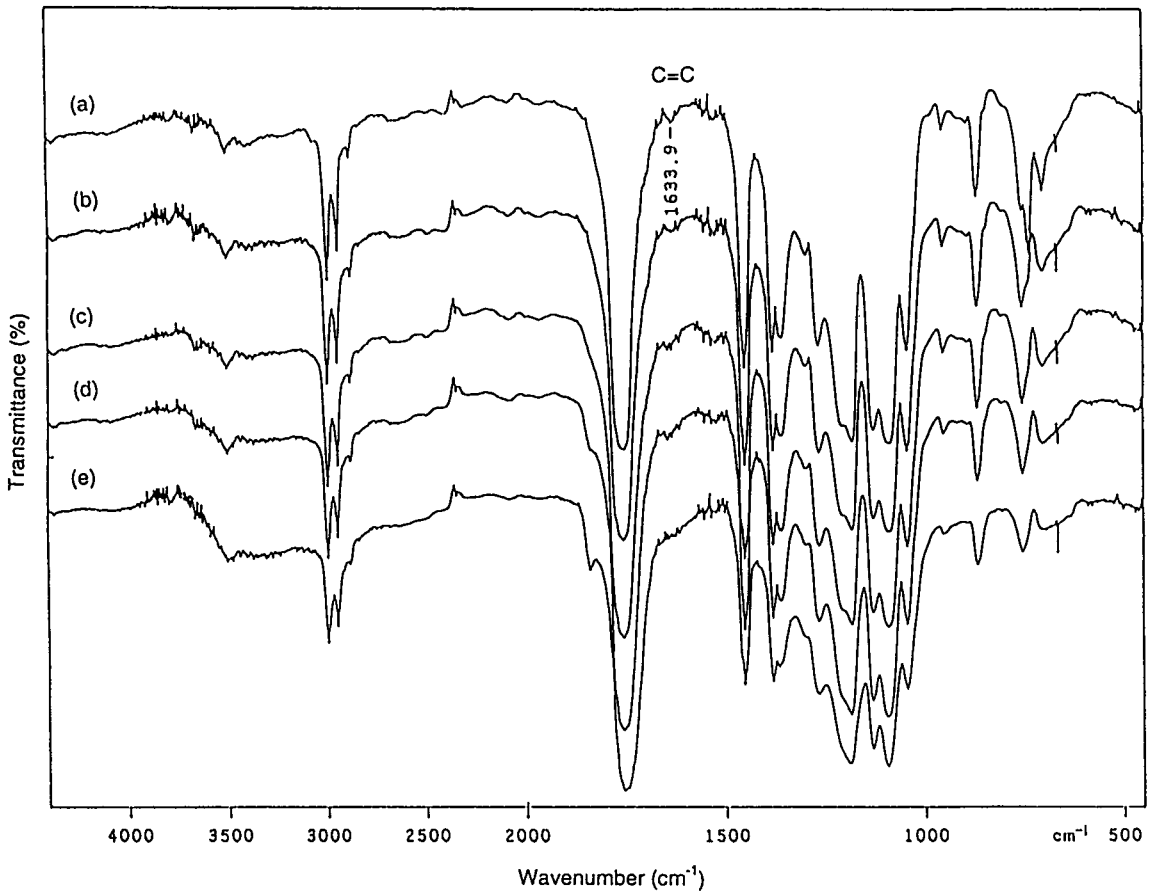


Figure 2. FTIR spectra of (a) acrylated polymer **4**, before UV irradiation, (b) after 3 min irradiation, (c) 5 min irradiation, (d) 10 min irradiation, and (e) 15 min irradiation.

not occur under our reaction conditions through deprotection. The catalytic hydrogenation thus appeared as a selective and facile method to remove the pendant benzyl protecting groups present in the copolymer.

The acrylic derivatives of the copolymer were synthesized by reacting PLA(Glc-Ser) copolymer with acryloyl chloride. The acrylate functionalization of PLA(Glc-Ser) was monitored by spectroscopic methods. Peaks at 6.4, 6.2, and 5.8 ppm in the ^{1}H NMR spectrum of the precipitated polymers after the reaction confirm the presence of acrylate groups as in Figure 1. The acrylate structure was again confirmed by the presence of a vinyl group at 128.4 and 131.5 ppm in the ^{13}C NMR spectrum. It should be noted that the peaks of acrylate groups are relatively small, because the number of serine units in the copolymer is rather low. The chemical structure of the acrylated polymers was also characterized using FTIR. From the FTIR results as shown in Figure 2, while the hydroxyl groups of the serine disappeared at 3510 cm^{-1} for acrylate, a vinyl group by acrylation was revealed at 1633.9 cm^{-1}. This was further confirmed by obtaining cross-linked polymers after UV irradiation for 2−10 min.

Properties of the PLA(Glc-Ser) Acrylated Polymer Networks. Polymer networks were prepared by different methods such as solution polymerization, bulk polymerization, and suspension methods. In the solution method, the acrylated polymer **4** was dissolved in CH_2Cl_2 and made a thin film using an applicator and irradiated by a 100 W medium-pressure mercury ultraviolet source to produce lactic acid/serine polymer networks. The photopolymerization was continued until gelation occurred. Durations of UV irradiation for these polymers ranged from 2 to 10 min, depending on the film thickness. The obtained network films were glassy and transparent. The cross-linking reaction was monitored using FTIR spectroscopy. A clean film was made on silicon wafer, and IR spectra were recorded before each 3 min irradiation time. The characteristic peaks of acrylate moiety at 1633.9 cm^{-1} were diminished and completely disappeared within 10 min irradiation as shown in Figure 2. The acrylated polymers gave a cross-linked network after UV irradiation for 5 min, depending on the thickness of the film.

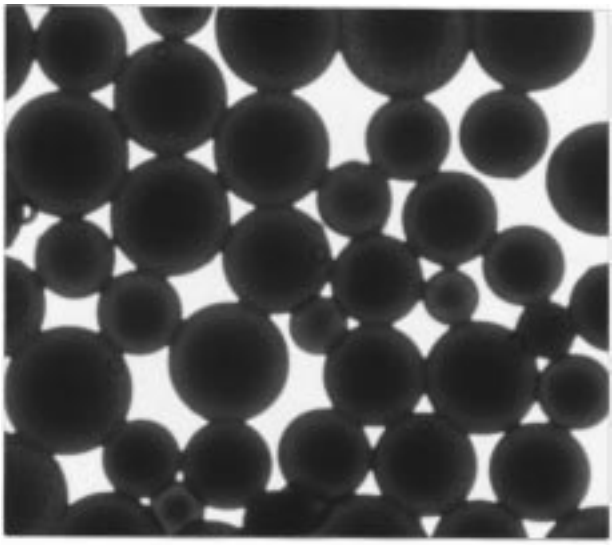
In bulk polymerization, PLA(Glc-Ser) acrylated derivative was mixed with excess of HEMA and UV irradiated for 5 min or until the polymer appeared solid. The polymer networks obtained were insoluble and swelled in DMSO and water. It is to be noted that insoluble networks were obtained even without adding any cross-linking agent such as EGDMA. However, the present study uses EGDMA for network formation.

Table 3 lists the bulk properties of PLA(Glc-Ser) networks. The swelling characteristics of a network are important in biomedical applications. Swelling affects solute diffusion, surface properties, mechanical properties, and surface mobility.[20] The degree of swelling of a polymer network or gel can be expressed as the swelling ratio. The degree of swelling of a gel depends on the pore size of the polymer network and the interaction between the polymer and the solvent.[21] Although the serine units of the polymer chain are hydrophilic, PLA is largely hydrophobic and is insoluble, giving a poor polymer interaction with water. This characteristic can be observed when the gels are swollen in water versus DMSO, a solvent in which the original copolymer [PLA-

Table 3. Bulk Properties of PLA(Glc-Ser) Polymer Networks

			solvent absorption, %			swelling ratio		
sample[a]	T_g (°C)	gel content	water	DMSO	$CHCl_3$	water	DMSO	$CHCl_3$
1	56.1	90	15	84	98	0.2	5	53
2	77.1	87	38	95	64	0.63	21	2

[a] Samples: (1) PLA(Glc-Ser), (2) PLA(Glc-Ser)/HEMA.



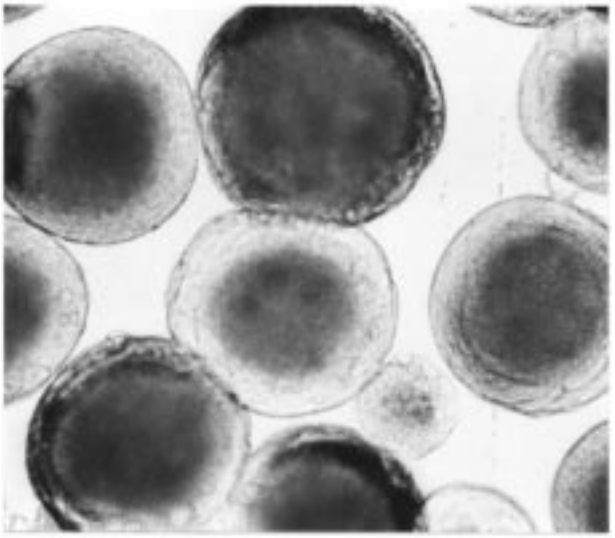


Figure 3. Representative optical micrographs of acrylated PLA(Glc-Ser) cross-linked beads produced by suspension polymerization: (a) before swelling, (b) after swelling in DMSO for 24 h.

(Glc-Ser)] is soluble. The networks are capable of absorbing a larger volume of DMSO and appear clear compared to the opaque gel which is formed when swollen in water as in Figure 3b.

The hydrophilicity of the modified PLA(Glc-Ser) **4** can be further increased by grafting hydrophilic monomers such as HEMA onto the polymer backbone. Preliminary results show that the hydrophilicity increased for the HEMA grafted polymer and are given in Table 3. Water absorption within the polymer networks was evaluated by differential weight measurements. The extent of water absorption measured was rather modest, though the maximum value being only 38%. These results indicate that the PLA(Glc-Ser)/HEMA networks synthesized could be of use as hydrogel applications in biomaterials use.

The thermal behavior of the polymer networks was examined by DSC. All the networks had no melting endotherms (T_m) because the acrylate groups were completely polymerized to form the cross-linked networks. The poly(HEMA) in PLA(Glc-Ser)/HEMA networks was phase mixed within the network bulk to a high degree, because one side of the PLA(Glc-Ser) was covalently grafted within the network and no separate melting point for poly HEMA. However, the DSC data displayed a glass transition temperature (T_g) due to the loose cross-linking by means of the less number of acrylated moieties in the polymer. The T_g values measured were slightly higher compared to the original polymer PLA(Glc-Ser) expected to be due to the increase in molecular weight during cross-linking.

The acrylated polymer **4** also underwent photopolymerization and cross-linking when the polymerization was conducted in a suspension system under UV light using 100 W medium-pressure mercury lamp. Cross-linked beads of almost uniform size range (25−170 μm) were obtained as shown in representative optical micrographs (Figure 3). The beads were not soluble in any of the solvents used for the copolymer. Swelling studies on the beads gave interesting results based on the difference in solvents. The beads were prepared in water medium, and the optical micrographs were taken immediately after collecting from the suspension medium showed opaque in nature (Figure 3a), while beads allowed to swell in DMSO for 24 h show a clear difference in the behavior of the polymers toward different solvents; the beads apparently become transparent (Figure 3b). The FTIR spectra of the both cross-linked network by solution polymerization and suspension polymerization obtained are quite similar, except for an increase in the peak height at 1730 cm^{-1} (which corresponds to carbonyl bonds). It is expected that EGDMA incorporation causes an increase in the number of carbonyl groups of the copolymer beads, which therefore results in an increase of the peak corresponding to these groups.

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