Synthesis and Characterization of Photo-Cross-Linked Polymers Based on Poly(L-lactic acid-*co*-L-aspartic acid)

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Introduction

Biodegradable polymers are used in the field of tissue engineering as scaffolds on which cells may grow and form tissue.[1] One objective in tissue engineering is to define polymer chemistries that mimic the *in vivo* environments in which cells naturally exist and to stimulate cells to form tissue. In particular, a tissue engineering scaffold needs to provide a three-dimensional structure for cell attachment and tissue regeneration while preserving the expression of differentiated cell phenotypes. The chemistry of the polymer can influence cell attachment and biocompatibility. Hydrogels have been a recent focus for the encapsulation of cells in tissue engineering.[2] 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 recently utilized photopolymerizations for the synthesis of biocompatible hydrogels based on poly(ethylene oxide). Photopolymerizations with these polymers produce minimal heat and require small quantities of photoinitiator, resulting in a biocompatible gel system which may be polymerized *in vivo*.[3]

Poly(α-hydroxy esters) such as poly(lactic acid) (PLA) are biodegradable polymers which have been used in medicine as sutures and in tissue engineering as polymer scaffolds.[1,4] PLA degrades into chemicals naturally found *in vivo* yet it has no functionality available for attachment of bioactive peptides or other further chemistry. The development of poly(lactic acid-*co*-lysine) (PLAL) was a step to introduce a functional group, the ϵ-amine of lysine, in PLA.[5] The number of functionalities was later increased by grafting amino acids from the ϵ-amine of lysine.[6]

The present work combines the use of degradable poly(α-hydroxy esters) and hydrogel photopolymerization technology to form gels based on methacrylated PLAL−ASP and their subsequent characterization.

Methods

Macromer Synthesis. PLAL was synthesized as previously described.[5] PLAL−ASP was subsequently synthesized by grafting poly(aspartic acid) side chains from the PLAL amine sites via the ring-opening polymerization of β-benzyl aspartate *N*-carboxyanhydride with aspartic acid contents of 25%.[6] The percent of aspartic acid of the polymer was determined by elemental analysis (Quantitative Technologies). All reagents were purchased from Aldrich and used as recieved for the methacrylate attachment. The percentage of aspartic acid units on PLAL−ASP which were converted to the methacrylate mixed anhydride was varied by the duration of the reaction at 37 and 60 °C. The graft copolymer was added to an excess of methacrylic anhydride to produce a 0.1% (w/v) solution of polymer to methacrylic anhydride. The reaction was heated to 37 or 60 °C in an oil bath under argon for 40 min to 1 day. The polymer was precipitated and washed by adding the methacrylic anhydride solution to a 10-fold excess of ether and vacuum filtering. The polymer was dried under vacuum overnight. The amount of methacrylate incorporated was determined by ^{1}H NMR (Varian 300 MHz) in DMSO-d_6. The percent of methacrylate groups which reacted with the carboxylic acid of aspartic acid was calculated by comparing the NMR integral intensities of the vinyl protons of the methacrylate group at 6.5−6.9 ppm and the lactide methine protons at 5.8 ppm.

UV Polymerization. Approximately 10 mg of PLAL−ASP was placed in a tissue culture insert (Falcon) with a diameter of 0.9 cm. A bulk polymerization was chosen over a solution polymerization since the polymer is soluble only in solvents which act as chain transfer agents and are cytotoxic in the case of cell encapsulation. Ten microliters of initiator solution containing 300 mg of DMPA 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 8 W LWUV lamp (GloMark Systems) for 5 min or until the polymer appeared solid. Gels had a diameter of 0.9 cm and a height of 1−4 mm. The gels were insoluble and swelled in water and DMSO.

Redox Polymerization. Ten milligrams of PLAL−ASP and 10 μL of HEMA were placed in a tissue culture insert with 200 μL of 0.1% (w/w) sodium metabisulfate and ammonium persulfate in PBS. The insert was then placed in an incubator at 37 °C and 5% CO_2 for 24 h.

Water Content. The cross-linked polymer was washed thoroughly 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 1 day in water or DMSO. Excess solvent was removed by lightly patting the gels with a towel, and swollen weights were obtained. Gel mass did not change over the 24 h in which characterization measurements and experiments were conducted.

Mechanical Testing. Mechanical measurements were made in uniaxial parallel-plate unconfined compression tests (Perkin-Elmer differential mechanical analyzer).[7,8] Gels were submerged in distilled water to maintain a hydrated state while a probe compressed the gel. The gel sample height required for strain calculations was determined by descending the calibrated probe until the sample was reached. Compression stresses with a defined creep rate were applied while measuring the resulting strain. Apparent moduli were determined by the slope of the stress versus strain at low strains. As strain reaches higher values, the specimen may undergo deformation in an unconfined compression test; therefore the slope of the stress−strain curve below a strain of 1.5% was used to determine the apparent modulus.

The stress recovery exhibited by the gels was tested by applying an unconfined uniaxial compression stress of 150 mN for 5 min followed by a stepwise reduction of the stress to 15 mN for 5 min. The gels relaxed more than 99% before 2 s, the time after which the instrument can respond with accurate strain measurements.

The elasticity was observed by repeated relaxation experiments in which a staircase stress was applied and removed.[9] Stress increments were applied with time for equilibration at each step until a maximum stress of 150 mN was reached, after which the stress was reduced in identical steps with equilibration after each reduction in stress. The apparent modulus was determined at each step by comparing the strain after equilibration to the stress applied.

Results and Discussion

Macromer Synthesis. PLAL−ASP with an aspartic acid content of 25% was synthesized in order to increase

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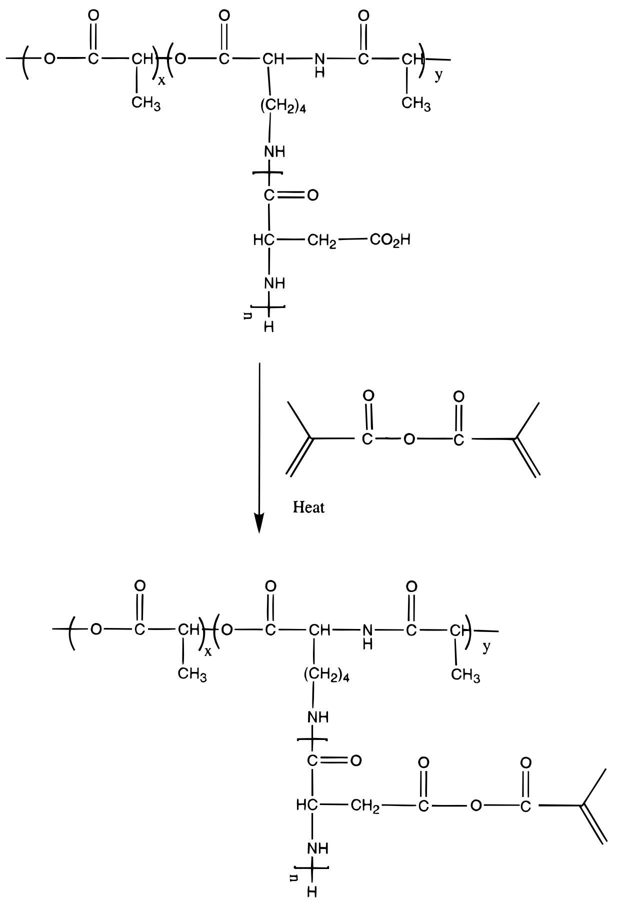
Scheme 1



the number of functional groups available for methacrylate attachment onto PLAL.[5] The PLAL−ASP polymers were reacted in an excess of methacrylic anhydride in order to form a mixed anhydride of the carboxylic acid of aspartic acid and the methacrylate group (Scheme 1). The percent of methacrylate groups attached to PLAL−ASP ranged from 5 to 22% depending on the reaction time and temperature. Five percent methacrylate attachment was obtained after a reaction time of 40 min at 37 °C while 22% was obtained after 3 h or more at 37 °C or 1 h at 60 °C (Figure 1). Table 1 shows the integration of the PLAL methine peak and the two methacrylate protons in columns two and three, respectively. The fourth column of the table is the ratio of a methacrylate proton to a PLAL proton. The ratio of the peak integral of the methacrylate protons and methine proton increases with a longer reaction time at 37 °C. The methacrylate to PLAL ratio is then multiplied by 0.75 to determine the percentage of the total polymer which was methacrylated since PLAL is only 75% of the total polymer as determined from aspartic acid elemental analysis.[5]

Gel Characterization. The swelling characteristics of a network are important in biomedical applications. Swelling affects solute diffusion, surface properties, mechanical properties, and surface mobility.[10] The degree of swelling of a polymer network or gel can be expressed as the equilibrium volume swelling ratio, Q, given by the volume of the gel in the swollen state versus the volume in the dry state:

$$Q = V_{\mathrm{swollen}}/V_{\mathrm{dry}}$$

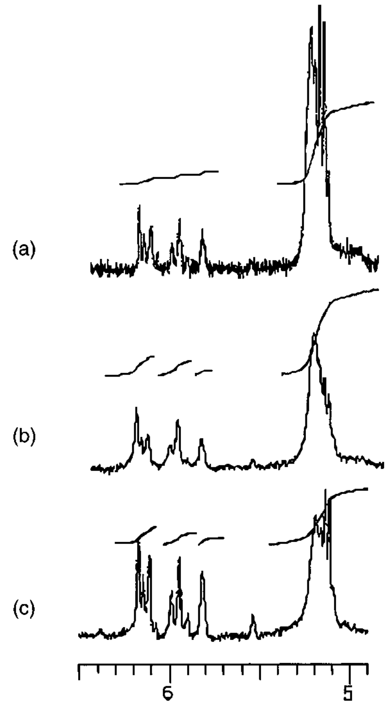


Figure 1. ^{1}H NMR spectra of vinyl and methine protons of PLAL−ASP reacted in methacrylic anhydride at 37 °C: (a) 40 min; (b) 1 h; (c) 3 h.

Table 1. Integration of ^{1}H NMR Peaks and Percent Methacrylation Calculated from Figure 1

spectrum in Figure 1	PLAL methine (5.2 ppm)	methacrylate protons (5.9−6.3 ppm)	ratio of protons methacrylate/ PLAL	% PLAL−ASP methacrylated
a	18.4	2.5	0.068	5.1
b	17.8	8.1	0.23	17.1
c	11.9	7.1	0.6	22.4

The density of PLAL−ASP needed for the calculation of polymer volume from weight was determined using the density of pLA (Sigma) and poly(aspartic acid) and the corresponding content of each in the polymer (1.25 g/mL).[11]

The average and standard deviation of the ratio Q was determined for networks with different concentrations of methacrylate groups. In the range of methacrylate group contents studied (5−22%), the degree of swelling remained constant with an average value of 5.53 ± 0.08 in PBS and 10.0 in DMSO. This suggests that the maximum number of methacrylate groups which form cross-links is $\leq$5%.

The degree of swelling of a gel is dependent on the pore size of the polymer network and the interaction between the polymer and the solvent.[11] Although the aspartic acid 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 macromer 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 with water.

Mechanical Properties. The apparent modulus determined by the initial slope of the stress−strain curve obtained from unconfined compression tests ranged from 1.4 to 3.1 kPa. The apparent modulus related inversely to methacrylate concentration, implying that as methacrylate percentage increased, the cross-linking density decreased. As a higher number of methacrylate

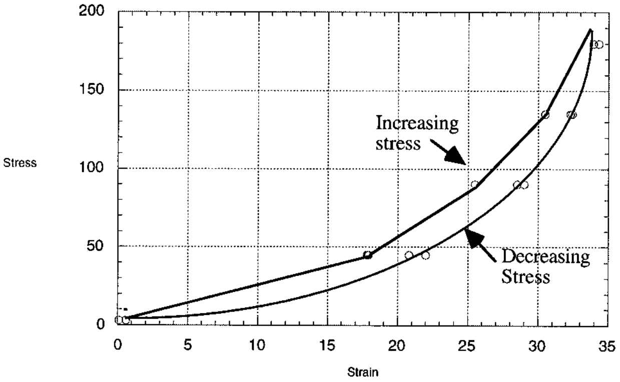


Figure 2. Results of staircase application and release of stress.

groups are attached to the aspartic acid side chain of PLAL–ASP, the methacrylate groups are in closer proximity to each other on a single chain and may react with each other forming loops or connections within a single polymer chain instead of reacting with a different polymer chain which would form a cross-link.

The apparent moduli were normalized with respect to the swollen polymer volume fraction. The swollen polymer fraction, $v_{2,s}$ is defined as the inverse of the equilibrium swelling ratio.[11] The normalized apparent moduli was calculated by

$$G_{\text{norm}} = \frac{G}{(v_{2,s})^{1/3}}$$

where G is the apparent modulus.[12] The normalized apparent moduli ranged from 2.47 to 5.39 kPa, a slightly larger range than for the moduli which were not normalized for differences in swelling.

The elasticity and apparent moduli were also tested in a dynamic stress experiment similar to those performed by Woo.[9] If the gel were perfectly elastic, the apparent moduli calculated at a step when the stress was increasing would be identical to the apparent moduli at the same step when the stress was decreasing. The results of the PLAL–ASP gels presented in Figure 2 show only a small amount of hysteresis, or decrease in the moduli as the stress is reduced. The upper curve in Figure 2 is the stress versus strain curve obtained as stress is stepwise increased while the lower curve is the result when stress is reduced. The small difference between these two curves implies that the gels exhibit viscoelastic properties. Both the static and dynamic stress–strain responses of the polymer network indicate that the gel is elastic and has mechanical integrity suitable for biomaterials/tissue engineering application.

For the applications of tissue engineering, particularly with cartilage, the compression strength of a material is of interest due to *in vivo* stresses experienced by cartilage in joints. In other applications such as tissue-engineered vessels, tracheae, or esophagus, the elasticity of the network is important due to the natural compliance of these tissues.

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