Published in final edited form as:

Biomacromolecules. 2008 October ; 9(10): 2784–2791. doi:10.1021/bm800557r.

Photocrosslinked PLA-PEO-PLA Hydrogels from Self-Assembled Physical Networks: Mechanical Properties and Influence of Assumed Constitutive Relationships

Naomi Sanabria-DeLong, Alfred J. Crosby, and Gregory N. Tew*

Department of Polymer Science & Engineering, University of Massachusetts, 120 Governors Dr., Amherst, MA 01003

Abstract

Poly(lactide) – *block* – poly(ethylene oxide) – *block* – poly(lactide) [PLA-PEO-PLA] triblock copolymers are known to form physical hydrogels in water, due to the polymer's amphiphilicity. Their mechanical properties, biocompatibility, and biodegradability have made them attractive for use as soft tissue scaffolds. However, the network junction points are not covalently crosslinked and in a highly aqueous environment these hydrogels adsorb more water, transform from gel to sol, and lose the designed mechanical properties. In this report, a hydrogel was formed by using a novel two step approach. In the first step end-functionalized PLA-PEOPLA triblock was self-assembled into a physical hydrogel through hydrophobic micelle network junctions, and then, in the second step, this self-assembled physical network structure was locked into place by photocrosslinking the terminal acrylate groups. In contrast to physical hydrogels, the photocrosslinked gels remained intact in phosphate buffered solution at body temperature. The swelling, degradation, and mechanical properties were characterized and demonstrated extended degradation time (~ 65 days), exponential decrease in modulus with degradation time, and tunable shear modulus (1.6 – 133 kPa) by varying concentration. We also discuss the various constitutive relationships (Hookean, Neo-Hookean, and Mooney-Rivlin) that can be used to describe the stress-strain behavior of these hydrogels. The chosen model and assumptions used for data fitting influences the obtained modulus values by as much as a factor of 3.5, demonstrating the importance of clearly stating one's data fitting parameters so that accurate comparisons can be made within the literature.

Introduction

Hydrogels have gained interest in the area of biomaterials for their many attractive qualities including high water content, porous structure, and tunable gelation conditions.^{1–4} These qualities allow the integration of such materials in the body as tissue scaffolds by offering structural support and allowing influx of cell metabolites and efflux of cell waste through their pores. Of even greater interest is to design hydrogels that can incorporate cells in a three dimensional structure while eventually degrading to leave behind only healthy tissue. To this effect, a number of researchers have investigated synthetic polymer hydrogels

*Corresponding Author.tew@mail.pse.umass.edu.

incorporating biocompatible hydrophilic poly(ethylene oxide) [PEO] segments along with biodegradable polyester domains, including poly(lactide) [PLA], poly(caprolactone) [PCL], and poly(glycolic acid) [PGA] just to name a few.^{2,5-14} In general ABA amphiphilic block copolymers form associative networks in water where the A block is hydrophobic and the B block is hydrophilic. This self-assembly is driven by the association of the hydrophobic endblocks into micellar structures, which are bridged by the water-soluble midblocks and form physically crosslinked networks. These physical hydrogels are attractive because no crosslinking agent is necessary and the gelation can be triggered by physically relevant stimuli (body temperature and pH). However, a number of groups have chemically crosslinked these polymers as well.¹⁵⁻¹⁸ Chemical crosslinking leads to a more permanent three-dimensional structure than the physically crosslinked counter-parts, but can still be degraded with time, and can be modified to incorporate proteins or adhesion peptides to increase the adhesion of cells to the scaffold.

While a variety of crosslinking techniques, polymer structures, and architectures have been used to synthesize biodegradable hydrogels, the corresponding mechanical properties are not as well characterized. This is unfortunate since the overall mechanical environment affects cell proliferation and growth.¹⁹⁻²¹ Cells typically bind to the extracellular matrix through surface receptors, but to migrate, traction forces must be generated. The underlying substrate must be able to withstand these traction forces so that the cell can grow and spread properly. One report has shown that cells can sense the restraining force of the underlying substrate and can respond by locally strengthening cytoskeleton linkages.²² This supports the work of many others who suggest that cells probe the stiffness of the surrounding environment to provide a feedback loop that can help determine the cell morphology, growth, and proliferation.²³⁻²⁸ Since the healthy survival of cells so greatly depends on the mechanical properties, it is important to consider the mechanical properties of both the target native tissue and the hydrogel when designing materials for cell scaffolds. This will allow stem cells to grow and differentiate into the desired cell type.²⁹

Mechanical properties of physically crosslinked hydrogels are typically characterized using shear rheometry, where the material is exposed to an oscillatory shear stress at various frequencies. This type of measurement leads to a determination of the storage modulus (G') and the loss modulus (G''), giving insight into the elastic and viscous components of the material, respectively. Mechanical properties of chemically crosslinked hydrogels are more typically characterized by measuring the stress in the material as strain is applied in compression. By implementing Hooke's Law ($\sigma = E\epsilon$, where σ is stress, ϵ is strain, and E is the Young's or elastic modulus), the slope of the linear region at low strains corresponds to the elastic modulus. However, Hooke's Law only applies to linearly elastic materials, while hydrogels typically have non-linear stress-strain responses. Models based on rubber networks such as a modified Neo-Hookean model³⁰⁻³² or a model defined by Mooney³³ and Rivlin³⁴ are more applicable for describing non-linear behavior and determining the modulus. In spite of this, many researchers still apply Hooke's Law to non-linear materials and thus the quantitative comparison of various hydrogel moduli is difficult.

In previous publications, we have reported varying degrees of stiffness of PLA-PEO-PLA physical hydrogels by manipulating the length of the PLA endblocks,³⁵ by changing the

physical crosslinks from amorphous to crystalline PLA,³⁶ and by varying the synthetic technique.³⁷ However, these hydrogels are dynamic (their crosslinks are not permanent); when exposed to a highly aqueous environment the gels continue to swell water and ultimately dissolve or precipitate out of solution losing all mechanical integrity. By using chemically crosslinked systems this problem is circumvented, but there is little control in the crosslinking reaction. While both physical gelation and photocrosslinking have been used to form hydrogels in the past, this work combines both approaches by first utilizing self-assembly through hydrophobic interactions, followed by chemical crosslinking. This novel approach allows for control of the network structure. More specifically, in this report we modified PLA-PEO-PLA triblock copolymer endgroups with acrylates so that the self-assembled structure could be locked in (permanently crosslinked) by initiating photocrosslinking with ultra-violet radiation after physical hydrogel formation. By characterizing the degree of swelling, the time necessary for complete degradation, and the mechanical properties while in compression, we assessed the viability of these materials as a tissue-engineering scaffold. We also discuss the relevance of common models for the hydrogel's constitutive relationship (Hookean, Neo-Hookean, or Mooney-Rivlin) and the importance of fully disclosing the method used and assumptions made for accurate quantitative comparison of results.

Experimental Section

Materials

3,6-Dimethyl-1,4-dioxane-2,5-dione (DL-lactide) (Sigma Aldrich) was recrystallized from ethyl acetate and sublimated prior to use. Tin (II) 2-ethylhexanoate catalyst (Alfa Aesar), PEO ($M_p = 8$ kDa provided by Sigma Aldrich, we previously performed MALDI-ToF analysis which showed the actual number average molecular weight to be 8.8 kDa), anhydrous toluene (99.8%, Sigma Aldrich), methacryloyl chloride (VWR - Alfa Aesar), and Irgacure 2959 (I2959) photoinitiator (Ciba) were used without further purification. Triethyl amine (TEA) was distilled over calcium hydride prior to use.

General Method for Triblock Polymerization

Telechelic PEO macroinitiator (60.5 g, 6.87 mmol, 1 equiv) was weighed into a dry 3-neck round bottom flask with a stir bar and attached to a condenser. The PEO was stirred and heated at 130 °C under nitrogen flow. The condenser was turned on and anhydrous toluene was added to the reaction mixture (approximate [PEO] = 50 mM). Tin (II) 2-ethylhexanoate (1.112mL, 3.44 mmol, 0.5 equiv) was added to the PEO, followed by immediate addition of DL-lactide (34.7 g, 0.240 mol, 35 equiv). The mixture was refluxed for 22 hours under nitrogen flow, removed from heat, quenched with methanol, diluted with tetrahydrofuran (THF), and precipitated using hexanes. The recovered white powder was separated with a filter funnel, collected, and dried under vacuum at room temperature. ¹H NMR (300 MHz, CDCl₃), δ : 5.12-5.19 (m), 3.64 (s), 1.48-1.59 (d), $M_n \sim 13,300$; GPC (DMF): PDI = 1.04.

PLA-PEO-PLA Acrylate End-Group Functionalization

PLA-PEO-PLA triblock copolymer (10.0 g, 0.760 mmol, 1 equiv) was weighed into a dried round-bottom flask, dissolved in toluene, and attached to a Dean-Stark trap with a

condenser. The system was evacuated and purged with nitrogen 3 times. The condenser was turned on and the solution was stirred and refluxed to azeotropically distill the solution. The distilled solution was cooled to room temperature and then placed in an ice bath.

Triethylamine (1.06 mL, 7.60 mmol, 10 equiv) was added dropwise, followed by dropwise addition of acryloyl chloride (0.617 mL, 7.60 mmol, 10 equiv), and stirred overnight.

Triethyl amine/hydrochloric acid salt was removed by filtration over filter paper, and the toluene was evaporated. The product was taken up in THF, passed through a plug of basic alumina, and precipitated in hexanes. ^1H NMR (300 MHz, CDCl_3), δ : 6.46-6.50 (d), 6.14-6.21 (m), 5.88-5.91 (d), 5.12-5.24 (m), 4.25-4.32 (m), 3.64 (s), 1.43-1.59 (d), $M_n \sim 12,420$; GPC (DMF): PDI = 1.06. ^1H NMR spectrum in supplemental, Figure S1.

Characterization of Polymer (^1H NMR and GPC)

^1H NMR spectra were recorded with a 400 MHz Bruker Spectrospin 300. Chemical shifts were expressed in parts per million using deuterated chloroform solvent protons as the standard. The average degree of polymerization (DP) was calculated by comparing the integration of the methyne peak of PLA to the integration of the methylene peak of the PEO block. The acrylate end group functionalization was quantitative as measured by ^1H NMR using integration of the acrylate protons to methylene PEO protons closest to the PLA ester linkage. Gel permeation chromatography (GPC) was performed with a Polymer Laboratories PL-GPC50 with 2 PLGel 5 μm Mixed-D columns, a 5 μm guard column, and a Knauer RI detector versus poly(styrene) standards. The eluent was N,N-dimethyl formamide with 0.01 M LiCl at 50 $^\circ\text{C}$. Example chromatogram shown in supplemental Figure S2.

Photocrosslinked Hydrogel Preparation

End-functionalized PLA-PEO-PLA (187 mg) was weighed into the wells of a 48-well cell culture plate. The plate was heated to 80 $^\circ\text{C}$ in a vacuum oven for 1.5 hour to melt a polymer film. After heating, the plate was cooled to room temperature. A 0.05% w/v I2959 solution was prepared by weighing 26 mg I2959 in a vial, adding 52 mL of phosphate buffered solution, and heating and sonicating to dissolve. For a 25% w/v hydrogel, 0.745 mL of 0.05% w/v I2959 solution was added to each well plate and allowed to swell into a physical gel over 3 - 4 days. After full swelling of the physical hydrogel, the well plates were irradiated with long-wave UV radiation (~ 365 nm) for 5 minutes, flipped upside-down (hydrogel thickness is approximately 8 mm), and irradiated for 5 more minutes to initiate the photocrosslinking. The hydrogel concentration was varied (10, 15, 25, 35, and 45% w/v) by adjusting the amount of dry polymer added to the well, while maintaining a constant volume of added photoinitiator solution. Picture of hydrogel shown in supplemental Figure S3.

Degradation and Swelling

Photocrosslinked polymer hydrogels were removed from the 48-well plates and placed into 24-well plates. The wells were filled with PBS and the immersed hydrogels were allowed to swell while in a 37 $^\circ\text{C}$ oven. The weight of the wet hydrogel (W_w) and the weight of the dried hydrogel (W_d , after drying in a vacuum oven to remove all water) was measured at various time points and used to calculate the swelling ratio, Q:

$$Q = \frac{W_w - W_d}{W_d} \quad (1)$$

The degradation of the hydrogels was determined through mass loss measurements defined by the equation below:

$$Mass \text{ Loss} = \left(\frac{m_{pi} - m_p}{m_{pi}} \right) \quad (2)$$

where m_{pi} is the initial dry weight of the polymer at time zero and m_p is the mass of the dried hydrogel after a defined swelling/degradation time. Each data point corresponds to the average of three measured hydrogels and time zero refers to the as-prepared hydrogel (25% w/v) before swelling in buffer solution. The surrounding buffer solution was replaced every 3 - 4 days to remove the acidic byproducts and maintain a constant pH of approximately 7.

Mechanical Properties in Compression

After removing the photocrosslinked hydrogels from the well plates, the rough top surface of the hydrogels was cut with a razor blade to give a cylindrical gel approximately 7.9 mm in height and 9.5 mm in diameter. Using an Instron with two flat plates, the gels were compressed at room temperature in air at a rate of 1 mm/min. Raw data (force vs. displacement) was converted to engineering stress and strain by using the initial dimensions of the gels. The moduli of the gels were measured by fitting the data to a Neo-Hookean model, a Mooney-Rivlin model, or an elastic Hookean model and are described in greater detail in the discussion. All data points are an average of 3 - 4 separate gels. Sample data for various degradation points and concentrations are shown in the Supplemental Figures S4 and S5.

Results and Discussion

PLA-PEO-PLA triblock copolymers were solution synthesized via ring-opening polymerization of DL-lactide with PEO macroinitiator ($M_n = 8800$ g/mol). The triblock copolymer was end-functionalized with acrylate groups yielding a photocrosslinkable polymer (Scheme 1) with a total degree of polymerization (DP) for PLA equal to 50 (DP = 25 per PLA endblock), a narrow molecular weight distribution (PDI = 1.06), and a total $M_n = 12.4$ kg/mol. The amphiphilicity of the triblock copolymer leads to self-assembly into a micellar network through hydrophobic interactions as illustrated in Figure 1a.^{38,39} Hydrophobic PLA is segregated to the micelle cores, while hydrophilic PEO either loops back to the same micelle or bridges to a neighboring micelle to create a network junction. However, this network structure is dynamic, meaning a polymer may pull out of one micelle core and insert into another, converting a crosslink to an ineffective loop or vice versa. As more water is added to the system, as would happen in the body, the distance between micelles increases, effectively lowering the density of junction points. Since there are no covalent bonds holding the junctions in place, at a certain critical concentration an associative network can no longer be formed. At this critical concentration this particular hydrogel system loses its mechanical integrity and is impractical for use as a cellular

scaffold. However, by introducing a photocrosslinkable moiety as described above, the self-assembled structure made by physical crosslinks can be captured once irradiated with longwave UV light that initiates the chemical crosslinking reaction (Figure 1b). Using this method, we can observe the differences between chemical and physical crosslinking in the same polymer hydrogel system.

The photocrosslinked PLA-PEO-PLA hydrogels were easily handled and remained intact when swollen in phosphate buffered saline solution (pH = 7.4) at body temperature (37 °C) for extended periods of time. In contrast, physical hydrogels would swell the excess solution until the system transforms from a gel to a sol. As shown in Figure 2 the 25% w/v photocrosslinked hydrogels started with a swelling ratio (Q) equal to approximately 3.5. When this gel was swollen in excess buffer, Q increased exponentially with time. Analogously, the amount of mass lost increased with time indicating degradation of the polymer hydrogel. Degradation occurs through hydrolysis of the ester linkages in the PLA blocks and can be described by a pseudo first-order kinetic equation.^{40,41} As this degradation occurred, the network opened up and allowed for more water to be swollen. Eventually, after about 52 days of swelling, the hydrogel was swollen enough that the gel was very difficult to handle and easily broken, in addition it fully degraded after approximately 63 days. The time-scale of degradation is longer than reports of similarly photocrosslinked PLA-PEO-PLA hydrogels,^{42,43} likely due to the self-assembled micellar structure prior to photocrosslinking and due to differences in molecular weights. This longer degradation time is useful for tissue engineering applications because it would allow the cells more time to grow into tissue before the scaffold, which offers structural support, is eliminated.

Because the differentiation of cells is highly influenced by its mechanical properties,²⁹ the performance of photocrosslinked PLA-PEO-PLA hydrogels was evaluated in compression. Typical stress strain curves from compression testing demonstrated the expected non-linear behavior exhibited by soft networks. To analyze the data the Neo-Hookean constitutive relationship for rubbers was utilized. In this model, the specific form of the strain energy function (U) is dependent on the first invariant of the deformation tensor (I_1) by the constant, C_1 :

$$U = C_1 (I_1 - 3)$$

$$U = C_1 (\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3)$$

Where λ_i is equal to the extension ratio in the i-principal direction, or more specifically, the length in the i-direction over the initial (pre-stressed) length in the i-direction. The extension ratio is related to the strain, ϵ , by the following expression: $\lambda = \epsilon + 1$. For the case of uniaxial compression and assuming the material to be incompressible:

$$\lambda_1^2 = \lambda^2, \lambda_2^2 = \lambda_3^2 = \lambda^{-1}$$

$$\lambda_1^2 \lambda_2^2 \lambda_3^2 = 1$$

By substituting into the strain energy expression and differentiating with respect to the extension ratio, an expression for stress (σ) is derived:

$$U = C_1 \left(\lambda^2 + \frac{2}{\lambda} - 3 \right)$$

$$\sigma = \frac{\pi U}{\pi \lambda} = 2C_1 \left(\lambda - \frac{1}{\lambda^2} \right) = 2C_1 \left[(\varepsilon + 1) - \frac{1}{(\varepsilon + 1)^2} \right] \quad (3)$$

where the single parameter C_1 is defined as half of the shear modulus, G ($C_1 = G/2$). The same relationship can be derived using a statistical thermodynamic approach in which the distribution of end-to-end distances between crosslinks is assumed to be Gaussian.³⁰⁻³² Figure 3 shows a stress-strain curve obtained for the 25% w/v gel along with its fit using this Neo-Hookean model. The shape of the curve is typical for all gels measured here (see SI). The fit is in excellent agreement with the data and predicts the observed non-linear behavior well, implying that the distribution of chains is indeed Gaussian and that there is little contribution from entanglements and looping chains to the overall network.

Using the Neo-Hookean fit, we observed the change in the gel stiffness as they were swollen and degraded with time in an aqueous environment (Figure 4). Initially, the 25% w/v hydrogels started with a shear modulus of ~ 64 kPa, but as they degraded, the shear modulus decreased exponentially to a value of ~ 7 kPa over a time scale of 35 days until a major drop in modulus occurred. The exponential decrease is in good agreement with the swelling data and the assumed first order kinetics for PLA hydrolysis.^{40,41} Crosslinks can only be broken through hydrolysis of ester linkages in the PLA blocks, and for first order kinetics, the PLA concentration decreases exponentially with time. The rate of hydrolysis is assumed to be proportional to the crosslink density (ρ_c), since as ester bonds are degraded, crosslinks are eliminated. This kinetic behavior is evident in the swelling data, as ρ_c and Q are inversely proportional ($Q \sim 1/\rho_c$), and accounts for the exponential decrease in modulus with time, as ρ_c and modulus are proportional ($G \sim \rho_c$).⁴⁴ After about 35 days, the modulus dropped significantly due to degradation. At this point, the crosslink density is likely low enough that network percolation is lost, leading to the abrupt decrease in hydrogel stiffness.

To probe the range of attainable moduli using photocrosslinkable PLA-PEO-PLA, hydrogels were prepared at various concentrations: 10, 15, 25, 35, and 45% (weight of polymer per volume of PBS). As gels were less concentrated they became softer, and as they were more concentrated they became stiffer, as expected. More specifically, there was a linear dependence of shear modulus on the hydrogel concentration (Figure 5). This effect can be explained by considering the hydrogel structure. As already described, the crosslinks in the network structure occur within the micelle core due to the formation of a physical gel prior to photocrosslinking, effectively equating the number of micelles to the number of junction points. A critical aggregation number (N_{agg}) of polymer chains is necessary to form a micelle. Assuming the critical N_{agg} remains constant and all micelles contribute to the network, then as the number of polymer chains (or the overall concentration) are increased linearly, so does the number of micelles (or crosslinks). Since modulus is directly proportional to the crosslink density, the linear relationship between modulus and concentration is well explained and demonstrates that the photocrosslinking process successfully locks in the pre-formed physical hydrogel structure.

Comparing Modulus Values Using Various Fitting Models

The compression behavior of these polymer hydrogels shows the typical non-linearity of soft rubbery materials and fits very well with the Neo-Hookean constitutive relationship described above. However, a more common and simple method used to determine modulus is a linear fit based on the Hookean constitutive relationship:

$$\sigma = E\varepsilon = E(\lambda - 1) \quad \text{Hookean Constitutive Relationship} \quad (5)$$

where stress is related to strain by the elastic modulus (E). While this relation only holds for the low strain, linear, elastic regime, it is often applied to hydrogel materials displaying non-linear behavior.

The data can also be fit using a more general two-parameter Mooney-Rivlin model.^{33,34} In this case, strain energy is linearly dependent on both the first and second invariants of the deformation tensor. Again, by assuming uniaxial compression and incompressibility of the material, then differentiating with respect to the extension ratio, the relationship can be simplified as shown below:

$$\begin{aligned} U &= C_1 (I_1 - 3) + C_2 (I_2 - 3) \\ U &= C_1 (\lambda_1^2 + \lambda_2^2 + \lambda_3^2) + C_2 (\lambda_1^2 \lambda_2^2 + \lambda_1^2 \lambda_3^2 + \lambda_2^2 \lambda_3^2 - 3) \\ \sigma &= \frac{\partial U}{\partial \lambda} = 2 \left(\lambda - \frac{1}{\lambda^2} \right) \left(C_1 + \frac{C_2}{\lambda} \right) \quad \text{Mooney-Rivlin Constitutive Relationship} \end{aligned} \quad (4)$$

The shear modulus in the Mooney-Rivlin constitutive relationship is two times the summation of the fit-parameters ($G = 2[C_1 + C_2]$). This model, like the Neo-Hookean model, also fits well for non-linear rubbery materials, but the second parameter allows for closer fits at high extension ratios. When fitting with the Mooney-Rivlin Model, C_1 was set to be equal to the C_1 determined from the Neo-Hookean fit, and C_2 was allowed to float to best fit the data.

Each of the above models fitted the data to various degrees; however, only the Neo-Hookean and Mooney-Rivlin models captured the non-linear behavior of the hydrogels in compression over the entire strain range. For photocrosslinked PLA-PEO-PLA hydrogels there was no linear elastic region, even at low strains (see inset of Figure 6), that could be adequately described by the Hookean relationship. Because of the gel's non-linear behavior, the modulus values obtained from fits using a Hookean relationship were dependent on the strain range to which the data was fit. At lower strains, the slope of the fitted line was less than that of higher strains. Since at higher strains more of the downward sloping non-linear region is taken into account, different values of the elastic modulus were measured. The extent of these effects were measured by fitting a Hookean relationship at 5, 10, and 15% strain with varying hydrogel concentrations, and the corresponding data is shown in Figure 7. All of the Hookean fits showed a linear dependence on hydrogel concentration as already described, but as the fitted strain range increased so did the modulus. Furthermore, for stiffer hydrogels the differences in moduli were more pronounced.

In order to compare the modulus values obtained from the Hookean model to those obtained from the Neo-Hookean and Mooney-Rivlin models, the shear modulus was converted to an elastic modulus using the following relation:

$$E=2G(1+\nu) \quad (6)$$

where ν is the Poisson's ratio, or the ratio of lateral tension to longitudinal compression. Most commonly, rubbers and gels are assumed to be incompressible with a Poisson's ratio equal to 0.5, which was assumed in the Neo-Hookean and Mooney-Rivlin models. Figure 8 shows the results using the above conversion with varying hydrogel concentrations. Again all the fits showed a linear dependence on concentration. Interestingly, the two-parameter Mooney-Rivlin fits determined modulus values that were almost identical to the one-parameter Neo-Hookean fit. Although the two-parameter model takes more of the high strain region into account, the one parameter model fits the data well enough that not much is gained in fitting with two parameters. Furthermore, although most rubbers are assumed to have a Poisson's ratio of 0.5, significantly lower Poisson's ratios have been measured for several hydrogels.⁴⁵⁻⁴⁷ For example, a Poisson's ratio as low as 0.33 has been reported for poly(vinyl alcohol) gels.⁴⁸ Therefore, if the actual Poisson's ratio of a material is not measured and is lower than that assumed, then a discrepancy between the real elastic modulus of the material and the measured elastic modulus will arise. The modulus values from all the fits are compared in Figure 9, and again illustrate that the value obtained is dependent on the fit used. The Hookean model at small strains (5%) gave the lowest elastic moduli, while the Neo-Hookean and Mooney-Rivlin models gave the highest moduli.

Conclusions

Physical hydrogel structures formed from PLA-PEO-PLA triblock copolymers were converted to chemically crosslinked hydrogels through end-group modification and subsequent photocrosslinking. Unlike physical hydrogels, the photocrosslinked systems remain intact in a highly aqueous environment giving better properties for tissue engineering applications. The modulus of the photocrosslinked gels decreased exponentially with time as they degraded for up to 35 days, after which a marked decrease in modulus was observed. We have also shown that the elastic modulus of the hydrogel can be tuned from 5 kPa to 400 kPa by controlling the polymer concentration.⁴⁹ This tunability will allow for better matching between the hydrogel scaffold material and soft target tissues.

This paper has also underscored how modulus values are influenced by the method of data analysis. The modulus of the hydrogel can be determined by choosing either a linear elastic Hookean model or the non-linear Neo-Hookean and Mooney-Rivlin models. However, the Hookean model can only be applied to small strain regions and does not capture the non-linear behavior of these soft rubbery materials. Moreover, our gels do not show a linear response even at very low strain, implying that the use of the linear Hookean model is inappropriate. We believe the Neo-Hookean model is the best for determining the hydrogel modulus, as it does capture the non-linear behavior and fits the data very well even at larger strains. The Mooney-Rivlin model is also applicable for these gels; however, the Neo-Hookean model is chosen as the most appropriate since only one parameter fit is needed and

can be statistically derived to give the fit parameter a physical meaning. Overall, greater care needs to be taken in reporting modulus values so that researchers know the conditions used for fitting the raw data. This will further help the field of biodegradable scaffolds so that more accurate correlations between mechanical properties and cell viability can be made between various literature reports.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

N.S.D. thanks the National Science Foundation (NSF) under Award No. DGE-0504485 for the Integrated Graduate Education and Research Traineeship (IGERT). N.S.D. also thanks Jeffrey A. Hubbell for hosting her at EPFL where this work was initiated, as well as André Van der Vlies and Dominique Rothenfluh for their guidance and assistance during her stay. G.N.T thanks the ARO and ONR Young Investigator programs, the NSF-CAREER, the 3M Nontenured faculty grant, and DuPont young faculty award for their support. We also thank the NSF for support of the Center for Hierarchical Manufacturing and the MRSEC (DMR-900488) for the use of characterization facilities.

References

- Hoffman AS. Adv. Drug Deliv. Rev. 2002; 54:3–12. [PubMed: 11755703]
- Kissel T, Li Y, Unger F. Adv. Drug Deliv. Rev. 2002; 54:99–134. [PubMed: 11755708]
- Lee KY, Mooney DJ. Chem. Rev. 2001; 101:1869–1879. [PubMed: 11710233]
- Kim B-S, Mooney DJ. TIBTECH. 1998; 16:224–230.
- Li S, Vert M. Macromolecules. 2003; 36:8008–8014.
- Molina I, Li S, Martinez MB, Vert M. Biomater. 2001; 22:363–369.
- Bae SJ, Joo MK, Jeong Y, Kim SW, Lee W-K, Sohn YS, Jeong B. Macromolecules. 2006; 39:4873–4879.
- Kwon K-W, Park MJ, Bae YH, Kim HD, Char K. Polymer. 2002; 43:3353–3358.
- Kim MS, Hyun H, Khang G, Lee HB. Macromolecules. 2006; 39:3099–3102.
- Lee HT, Lee DS. Macromol. Res. 2002; 10:359–364.
- Lee DS, Shim MS, Kim SW, Lee H, Park I, Chang T. Macromol. Rapid Commun. 2001; 22:587–592.
- Zhong Z, Dijkstra P, Feijen J, Kwon YM, Bae YH, Kim SW. Macromol. Chem. Phys. 2002; 203:1797–1803.
- Fujiwara T, Mukose T, Yamaoka T, Yamane H, Sakurai S, Kimura Y. Macromolecular Bioscience. 2001; 1:204–208.
- Shim WS, Yoo JS, Bae YH, Lee DS. Biomacromolecules. 2005; 6:2930–2934. [PubMed: 16283710]
- Tirelli N, Lutolf MP, Napoli A, Hubbell JA. Reviews in Molecular Biotechnology. 2002; 90:3–15. [PubMed: 12069046]
- Lutolf MP, Hubbell JA. Nature Biotechnology. 2005; 23:47–55.
- Nuttelman CR, Rice MA, Rydholm AE, Salinas CN, Shah DN, Anseth KS. Progress in Polymer Science. 2008; 33:167–179. [PubMed: 19461945]
- Elisseff J, Anseth K, Sims D, McIntosh W, Randolph M, Langer R. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96:3104–3107. [PubMed: 10077644]
- Brandl F, Sommer F, Goepferich A. Biomater. 2007; 28:134–146.
- Huang S, Ingber DE. Nat. Cell Bio. 1999; 1:131–138.
- Georges PC, Janmey PA. Journal of Applied Physiology. 2005; 98:1547–1553. [PubMed: 15772065]

22. Choquet D, Felsenfeld DP, Sheetz MP. Cell. 1997; 88:39–48. [PubMed: 9019403]
23. Wang HB, Dembo M, Wang YL. Am. J. Physiol. Cell Physiol. 2000; 279:C1345–C1350. [PubMed: 11029281]
24. Engler AJ, Griffin MA, Sen S, Bonnemann CG, Sweeney HL, Discher DE. J. Cell Bio. 2004; 166:877–887. [PubMed: 15364962]
25. Pelham RJ, Wang Y-L. Proceedings of the National Academy of Sciences of the United States of America. 1997; 94:13661–13665. [PubMed: 9391082]
26. Pelham RJ, Wang Y-L. Molecular Biology of the Cell. 1999; 10:935–945. [PubMed: 10198048]
27. Kim B-S, Nikolovski J, Bonadio J, Mooney DJ. Nature Biotechnology. 1999; 17:979–983.
28. Chicurel ME, Chen CS, Ingber DE. Current Opinion in Cell Biology. 1998; 10:232–239. [PubMed: 9561847]
29. Engler AJ, Sen S, Sweeney HL, Discher DE. Cell. 2006; 126:677–689. [PubMed: 16923388]
30. Wall FT. Journal of Chemical Physics. 1942; 10:485–488.
31. Flory PJ, Rehner J. J. Journal of Chemical Physics. 1943; 11:512–520.
32. Treloar LR. G. Transactions of the Faraday Society. 1943; 39:241–246.
33. Mooney M. Journal of Applied Physics. 1940; 11:582–592.
34. Rivlin RS. Philosophical Transactions of the Royal Society of London. Series A, Mathematical and Physical Sciences. 1948; 241:379–397.
35. Aamer KA, Sardinha H, Bhatia SR, Tew GN. Biomater. 2004; 25:1087–1093.
36. Sanabria-DeLong N, Agrawal SK, Bhatia SR, Tew GN. Macromolecules. 2006; 39:1308–1310.
37. Sanabria-DeLong N, Agrawal SK, Bhatia SR, Tew GN. Macromolecules. 2007; 40:7864–7873.
38. Agrawal SK, Sanabria-DeLong N, Tew GN, Bhatia SR. Macromolecules. 2008; 41:1774–1784.
39. Agrawal SK, Sanabria-DeLong N, Tew GN, Bhatia SR. Langmuir. 2007; 23:5039–5044. [PubMed: 17397197]
40. Metters AT, Anseth KS, Bowman CN. Journal of Physical Chemistry B. 2001; 105:8069–8076.
41. Metters AT, Anseth KS, Bowman CN. Polymer. 2000; 41:3993–4004.
42. Anseth KS, Metters AT, Bryant SJ, Martens PJ, Elisseeff JH, Bowman CN. Journal of Controlled Release. 2002; 78:199–209. [PubMed: 11772461]
43. Bryant SJ, Bender RJ, Durand KL, Anseth KS. Biotechnology and Bioengineering. 2004; 86:747–755. [PubMed: 15162450]
44. Flory PJ, J. Rehner J. Journal of Chemical Physics. 1943; 11:521–526.
45. De SK, Aluru NR, Johnson B, Crone WC, Beebe DJ, Moore J. Journal of Microelectromechanical Systems. 2002; 11:544–555.
46. Takigawa T, Ikeda T, Takakura Y, Masuda T. Journal of Chemical Physics. 2002; 117:7306–7312.
47. Li Y, Hu Z, Li CJ. Appl. Poly. Sci. 1993; 50:1107–1111.
48. Urayama K, Takigawa T, Masuda T. Macromolecules. 1993; 26:3092–3096.
49. The elastic moduli were converted from shear moduli values assuming an incompressible isotropic material ($G = 1.6 - 133 \text{ kPa}$).

Scheme 1. Acrylation of PLA-PEO-PLA

PLA-PEO-PLA is reacted with acryloyl chloride using triethylamine as a basic catalyst to yield the acrylate end-functionalized triblock copolymer. Hydrophobic PLA is illustrated in green while hydrophilic PEO is in blue.

Figure 1. Self-Assembled Physical Hydrogel Structure and Conversion to Chemical Hydrogel
The physical crosslinks are dynamic as illustrated by the dashed lines around the micellar cores (left), but once photocrosslinked the junction points are permanent, as shown using solid lines around the micelle cores (right).

Figure 2. Swelling and degradation of photocrosslinked PLA-PEO-PLA

25% w/v hydrogels were swollen in PBS. Changes in swelling ratio (Q) and % mass loss were measured. Q increases exponentially with time as shown by the black fitted line.

Figure 3. Typical Stress versus Strain Curve in Compression

25% w/v photocrosslinked PLA-PEO-PLA before degradation. Stress curve is non-linear and typical of soft rubbery materials as well as all of the gels measured in this report.

Figure 4. Shear modulus of degrading hydrogel

The modulus of a 25% w/v hydrogel decreases exponentially until a critical degradation time when the modulus drops precipitously.

Figure 5. Shear modulus with varying hydrogel concentration

The modulus is linearly dependent on concentration as would be predicted for physical hydrogels.

Figure 6. Typical Stress versus Strain Curve with Hookean and Neo-Hookean Model Fits

The Hookean model (shown in green, blue, and red at 5, 10, and 15% strain range, respectively) does not capture the non-linearity in the stress-strain curve, while the Neo-Hookean fit (in black) does. The inset zooms in on the small strain region and shows that even at low strains, the curve is non-linear.

Figure 7. Elastic Modulus vs. Concentration using Hookean Fits with various strain ranges
Using larger strain ranges for fitting with a Hookean model leads to larger modulus.

Figure 8. Elastic Modulus vs. Concentration using Rubber Models

The two-parameter Mooney-Rivlin model calculates the same modulus as the one-parameter Neo-Hookean model (Note that these models assume incompressibility and a Poisson's ratio of 0.5).

Figure 9. Comparing Elastic Modulus using Various Fits

The reported modulus values are highly dependent on which constitutive relationship is assumed.