Published in final edited form as:

Macromol Mater Eng. 2013 June 1; 298(6): 699–703. doi:10.1002/mame.201200119.

Characterization of Thiol-Ene Crosslinked PEG Hydrogels

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Abstract

The properties of synthetic hydrogels can be tuned to address the needs of many tissue-culture applications. This work characterizes the swelling and mechanical properties of thiol-ene crosslinked PEG hydrogels made with varying prepolymer formulations, demonstrating that hydrogels with a compressive modulus exceeding 600 kPa can be formed. The amount of peptide incorporated into the hydrogel is shown to be proportional to the amount of peptide in the prepolymer solution. Cell attachment and spreading on the surface of the peptide-functionalized hydrogels is demonstrated. Additionally, a method for bonding distinct layers of cured hydrogels is used to create a microfluidic channel.

Keywords

biomaterials; biopolymers; hydrogels; mechanical properties; microfluidics

1. Introduction

The extracellular matrix (ECM) plays a key role in defining the cellular microenvironment, providing both physical and chemical cues that are vital to maintaining proper cellular function.^[1] Matrigel and other naturally derived ECM materials are commonly used for scientific research and tissue engineering to mimic the cell microenvironment; however, the complex composition of Matrigel^[2] and the potential for lot-to-lot variation has motivated the development of well defined synthetic materials that can be formulated to mimic specific chemical and mechanical cues present in ECM microenvironments.

For example, the role that mechanical cues play in the regulation of cell behavior is becoming increasingly evident.^[3] The mechanical properties of human tissues vary widely from brain (0.1–1 kPa) to bone ($\approx 10^6$ kPa),^[1b] making it particularly difficult to use only one type of biomaterial to test the entire range of mechanical microenvironments experienced by cells. For example, synthetic hydrogels tend to be limited to lower mechanical stiffness due to the high degree of water incorporation into the swollen network structure.

Thiol-ene coupling chemistry was recently demonstrated by Anseth and coworkers to be an efficient means of producing poly(ethylene glycol) (PEG) hydrogels with tunable properties for cell culture applications.^[4] The thiol-ene polymerization occurs through a step-growth mechanism that produces a more homogeneous network structure than the chain growth mechanism that defines the free radical polymerization of vinyl functionalized polymers.^[5] The homogeneous nature of the formed network leads to a hydrogel with more uniform and predictable properties. In this work, we look to further characterize thiol-ene crosslinked PEG hydrogels, both in terms of mechanical and chemical properties, and to examine the potential to use these materials to probe higher ranges of hydrogel stiffness. We show that a single crosslinking step can produce a hydrogel that has a compressive modulus in excess of 600 kPa. Further, we use the step-growth mechanism to bond a hydrogel containing excess thiol functionality to a hydrogel containing excess norbornene functionality. The bonding method has several potential applications and herein we demonstrate the fabrication of a microfluidic device to demonstrate the utility of the technique.

2. Experimental Section

Multi-arm PEG norbornene molecules were synthesized using methods described previously.^[4] The PEG starting material was purchased from JenKem Technology USA and all other materials used in the synthesis were purchased from Sigma-Aldrich. Multiarm PEG thiols were purchased from Laysan Bio and used as received. The CGGGRGDSP peptide was synthesized on rink amid resin using Fmoc-protected amino acids from Anaspec. Irgacure 2959 was purchased from Ciba.

Hydrogels were made by weighing out dry monomer precursors in a 1:1 functional group ratio and then adding deionized water and a solution of Irgacure 2959 in deionized water. The final concentration of Irgacure in the precursor solution was 0.05% w/v. The solution was thoroughly mixed using a vortex mixer. For swelling studies, precursor solution (70 μ L) was

placed in custom molds consisting of a Teflon sheet with 7.6mm wide, 1.5mm deep wells. The solution was then crosslinked with a 365nm UV light ($4\text{mW}\cdot\text{cm}^{-2}$) for 100 s. The crosslinked gels were then transferred into well plates containing 1mL of PBS solution. The disks were allowed to swell for 24 h prior to measurement. To determine the swelling, the hydrogel disk diameters were measured and the disks were weighed after excess buffer was removed using weighing paper. The disks were then placed in well plates with deionized (DI) water. The disks were soaked in DI water over the course of 24 h, with the solution being replaced every 8 h in order to remove salt from the hydrogels. The hydrogels were then frozen and lyophilized in order to determine the dry weight of the gels.

Mechanical testing samples were prepared using the same solutions, except a larger mold was used to form the disks. For the mechanical testing samples, 360 μL of precursor solution was added to a 13.5mm wide, 2.5mm deep well and crosslinked for 100 s. The disks were then placed in well plates with 1.5mL of solution and allowed to swell for 24 h. The disk thickness and diameter were then measured prior to mechanical testing. Compression testing was performed on an MTS Insight testing apparatus using a constant deformation rate of $0.1\text{mm}\cdot\text{min}^{-1}$.

The CGGGRGDSP peptide was made using a CS Bio automated peptide synthesizer. The amount of peptide ligand incorporated into the hydrogel discs was determined using a modified Micro BCA technique (Pierce), as previously described.^[6]

3. Results and Discussion

Thiol-ene hydrogel networks were created with varying monomer concentration, molecular weight, and number of functional groups. Compression modulus increased with increasing monomer concentration in the prepolymer solution, increasing number of functional groups per monomer, and decreasing monomer molecular weight (Figure 1A). A 25 wt% 8-arm PEGNB ($M_w = 10\text{ kDa}$)/4 arm PEG thiol ($M_w = 10\text{ kDa}$) formulation had the highest modulus of the combinations that was tested (630 kPa) while a 5 wt% 4-arm PEGNB ($M_w = 20\text{ kDa}$)/PEG dithiol ($M_w = 3.4\text{ kDa}$) had the lowest modulus of the tested materials (7 kPa). While these values represent a wide range of extremes in stiffness, both lower and higher values could likely be achieved by further modifying the variables of molecular weight, degree of functionality, and wt% of the precursor solution. Hydrogel swelling was found to be inversely related to hydrogel stiffness (Figure 1B). Hydrogel swelling was further characterized by varying the molecular weight of the 4-arm PEGNB and the swelling was found to increase with increasing PEGNB molecular weight (Figure 2A). Swelling of a 20 kDa PEGNB/3.4 kDa PEG dithiol did not significantly change when the photoinitiator concentration was varied from 0.5 to 0.005 wt% (Figure 2B).

The increase in swelling observed with increased molecular weight and decreasing solids content in the prepolymer solution follow similar trends observed in kinetic growth hydrogels. It is not surprising that the inverse relationship between hydrogel swelling and stiffness is the same regardless of the chemical crosslinking mechanism, as swelling is a response to osmotic pressure extending the chains of the polymer network. If a polymer shows low resistance to osmotic pressure and swells, then it is equally likely that the

polymer network will be deformed when external forces are applied. Swelling is also related to the mesh size of the polymer network, and we can thus speculate that stiffer hydrogels may inhibit mass transfer in applications where cells are encapsulated in the hydrogel.

In addition to mechanical properties, presentation of immobilized biochemical signals on the ECM is also important in many biological applications. As has been shown in previous work, peptide ligands can be incorporated into PEGNB hydrogel networks via the sulfhydryl group of an exposed cysteine residue. We used the integrin binding peptide sequence CGGGRDGSP to test the efficiency with which small peptide ligands are incorporated into PEGNB hydrogels using thiol-ene chemistry. The amount of peptide incorporated into the hydrogel network was found to be proportional to the amount of peptide in the prepolymer solution, with higher incorporation efficiency at lower peptide concentrations (Figure 3A). Peptide incorporation into the hydrogel was not quantitative, likely due to factors such as mass transfer limitations and disulfide bond formation. Given that peptide incorporation is not quantitative, it is important to measure the peptide incorporated into the crosslinked hydrogel after crosslinking in order to report accurate ligand concentrations. The peptide ligand enabled NIH3T3 fibroblasts to attach to the surface of a hydrogel functionalized with the integrin binding peptide, and the gel had sufficient ligand density to allow the cells to spread on the surface of the gel (Figure 3B).

In addition to promoting a homogeneous network structure, the step-growth mechanism also enables hydrogel layers to be bonded together by using excess thiol groups in one layer and excess norbornene groups in the opposite layer. Placing the two gels in contact and exposing the gels to UV light in the presence of photoinitiator can then bond the two layers together. A microfluidic device was generated by using a 1.3:1 molar excess of thiol monomer in one layer and 1.3:1 molar excess of norbornene monomer in the opposing layer (Figure 4). After the device was formed, the channel was filled using a syringe at one inlet to inject dye and a needle at the outlet to vent air. Hydrogel microfluidic devices are already being used to create 3D cellular microenvironments^[7] and the new bonding technique presented here should enable the generation of 3D microenvironments with asymmetrical chemical and mechanical properties. Bonding of layers with differing swelling properties might also be used to generate hydrogels that fold into more complex 3D geometries.^[8]

4. Conclusion

The mechanical properties of thiol-ene crosslinked PEG hydrogels can be formulated to achieve a wide range of compressive moduli. The stiffest hydrogels were formed using low-molecular-weight monomers in conjunction with a high degree of functionality and a high monomer loading in the prepolymer solution. Stiff hydrogels derivatized with an integrin binding peptide were able to allow cells to attach and spread on the surface. The peptide incorporated into the hydrogels was proportional to the concentration of the peptide in the prepolymer solution. The ability to bond individual hydrogel layers enables creation of microfluidic channels, and opens up the possibility of creating chemically and mechanically asymmetric cellular microenvironments.

Acknowledgments

The authors acknowledge support from the National Science Foundation (CAREER award 0745563) and the National Institutes of Health (R01HL093282 and T32 DC009401) and thank Alexander Laperle and Professor Kristyn Masters for assistance with the mechanical testing.

References

1. a) Bryant SJ, Bender RJ, Durand KL, Anseth KS. *Biotechnol Bioeng.* 2004; 86:747. [PubMed: 15162450] b) Engler AJ, Sen S, Sweeney HL, Discher DE. *Cell.* 2006; 126:677. [PubMed: 16923388] c) Gobaa S, Hoehnel S, Roccio M, Negro A, Kobel S, Lutolf MP. *Nat Methods.* 2011; 8:949. [PubMed: 21983923] d) Spradling A, Drummond-Barbosa D, Kai T. *Nature.* 2001; 414:98. [PubMed: 11689954]
2. Hansen KC, Kiemele L, Maller O, O'Brien J, Shankar A, Fornetti J, Schedin P. *Mol Cell Proteomics: MCP.* 2009; 8:1648.
3. a) Gilbert PM, Havenstrite KL, Magnusson KE, Sacco A, Leonardi NA, Kraft P, Nguyen NK, Thrun S, Lutolf MP, Blau HM. *Science.* 2010; 329:1078. [PubMed: 20647425] b) Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, Bencherif SA, Rivera-Feliciano J, Mooney DJ. *Nat Mater.* 2010; 9:518. [PubMed: 20418863] c) Nemir S, West JL. *Ann Biomed Eng.* 2010; 38:2. [PubMed: 19816774] d) Park JS, Chu JS, Tsou AD, Diop R, Tang Z, Wang A, Li S. *Biomaterials.* 2011; 32:3921. [PubMed: 21397942] e) Pek YS, Wan AC, Ying JY. *Biomaterials.* 2010; 31:385. [PubMed: 19811817]
4. Fairbanks BD, Schwartz MP, Halevi AE, Nuttelman CR, Bowman CN, Anseth KS. *Adv Mater.* 2009; 21:5005.
5. Fairbanks BD, Scott TF, Kloxin CJ, Anseth KS, Bowman CN. *Macromolecules.* 2009; 42:211. [PubMed: 19461871]
6. Impellitteri NA, Toepke MW, Lan Levengood SK, Murphy WL. *Biomaterials.* 2012; 33:3475. [PubMed: 22322198]
7. a) Bettinger CJ, Borenstein JT. *Soft Matter.* 2010; 6:4999. b) Cross VL, Zheng Y, Won Choi N, Verbridge SS, Sutermaster BA, Bonassar LJ, Fischbach C, Stroock AD. *Biomaterials.* 2010; 31:8596. [PubMed: 20727585] c) Cuchiara MP, Allen AC, Chen TM, Miller JS, West JL. *Biomaterials.* 2010; 31:5491. [PubMed: 20447685] d) Paguirigan A, Beebe DJ. *Lab Chip.* 2006; 6:407. [PubMed: 16511624]
8. Klein Y, Efrati E, Sharon E. *Science.* 2007; 315:1116. [PubMed: 17322058]

Figure 1. Mechanical properties of thiol-ene crosslinked PEG hydrogels. Compressive modulus of hydrogel disks with varying formulations (A), swelling ratio of hydrogel disks with varying formulation (B), and a photograph of a 250 mL glass beaker sitting on top of a hydrated 25 wt% 10 kDa 8-arm PEG norbornene/10 kDa 4-arm thiol hydrogel, which retains its swollen form (C).

Figure 2. Thiol-ene PEG hydrogel swelling increases with increasing molecular weight of monomer (A). Hydrogel swelling was not found to vary as a function of photoinitiator concentration under the conditions that were tested (B).

Figure 3. Efficiency of CGGGRGDSP peptide incorporation into PEG hydrogels crosslinked using thiol-ene chemistry (A). NIH 3T3 fibroblasts can spread on the surface of stiff hydrogels that contain the peptide CGGGRGDSP (B).

Figure 4. Creating layers with excess functional groups can be used to bond PEG hydrogels together, which can be used to form microfluidic devices (bottom right) and other complex structures.