Published in final edited form as:

J Biomed Mater Res A. 2010 February ; 92(2): 441–450. doi:10.1002/jbm.a.32353.

The Effects of Monoacrylated Poly(Ethylene Glycol) on the Properties of Poly(Ethylene Glycol) Diacrylate Hydrogels Used for Tissue Engineering

Jeffrey A Beamish, M.S.^a, Junmin Zhu, Ph.D.^a, Kandice Kottke-Marchant, M.D./Ph.D.^{a,b}, and Roger E. Marchant, Ph.D.^{a,*}

Jeffrey A Beamish: jeffrey.beamish@case.edu; Junmin Zhu: junmin.zhu@case.edu; Kandice Kottke-Marchant: marchak@ccf.org; Roger E. Marchant: roger.marchant@case.edu

^a Department of Biomedical Engineering, Case Western Reserve University, 10900 Euclid Ave., Wickenden 309, Cleveland, OH 44106, USA (Tel.: +1-216-368-2312, fax: +1-216-368-4969)

^b Division of Pathology and Laboratory Medicine, Cleveland Clinic, L21, 9500 Euclid Ave., Cleveland, OH 44195, USA (Tel.: 216-444-2484, fax: +1-216-445-9444)

Abstract

This study investigated the effects of poly(ethylene glycol) monoacrylate (PEGMA) on the properties of poly(ethylene glycol) diacrylate (PEGDA)-co-PEGMA hydrogel networks. The PEGMA materials utilized were similar to ligand-linked materials typically copolymerized with PEGDA for use as tissue engineering scaffolds. 5–20% (wt/wt) PEGDA (6 kDa) and 0–20% (wt/wt, 0–43 mM) PEGMA (5 kDa) were copolymerized by photo-initiated free radical polymerization and the mass swelling ratio and shear modulus of the resulting hydrogels were determined. Increasing the pre-polymerization concentration of PEGMA decreased the swelling ratio by up to 42±1.6% and increased the shear modulus by up to 167±29.3%, suggesting that PEGMA enhanced gel cross-linking. Analysis of the effective number of cross-linked chains per PEGDA, calculated independently from swelling and mechanical data, indicated each PEGDA participated in more cross-links as PEGMA was added. The results suggest that PEGMA-co-PEGDA gels can be formed with higher concentrations of PEGMA-tethered ligands than previously reported allowing the formation of scaffolds with a rich diversity of biological functionalities without sacrificing the integrity of the gel network.

Keywords

hydrogel; poly(ethylene glycol); scaffold; tissue engineering; polymer network

Introduction

Poly(ethylene glycol) (PEG) based hydrogels have attracted broad interest as a scaffold material for tissue engineering applications^{1–3}. The PEG backbone of these materials resists the adsorption of exogenous proteins and does not support cell attachment. However, the inclusion of biological ligands allows cell interactions with the scaffold that can be controlled quantitatively. PEG networks have been formed using a variety of approaches including radiation cross-linking, modification with fumarate, acrylate or methacrylate groups followed by free radical photopolymerization, and by linking sulfone terminated multiarm PEG with disulfhydryl cross-linkers via Michael addition^{1–4}.

*Corresponding author. Tel.: +1-216-368-3005; fax: +1-216-368-4969; e-mail: roger.marchant@case.edu.

Photopolymerization, including photolithography, of PEG di(meth)acrylate (PEGDA) has been employed specifically for tissue engineering applications because these gels can be formed readily *in situ*⁵. This allows scaffolds to be engineered in a variety of 3-dimensional structures and with gradients of biological agents. Proteolytically or hydrolytically degradable PEGDA derivatives have been synthesized to enhance the utility of this system as a scaffold material further^{6,7}. Acrylation followed by photoinitiated chain polymerization also has been used widely to form networks from a variety of biological and synthetic materials used for tissue engineering applications^{8–11}.

The network structure of PEGDA hydrogels is distinct from traditional polymer networks that are formed by cross-linking preformed polymer chains or by copolymerizing monomer with a small fraction of a cross-linker. PEGDA hydrogels are formed typically from a single component that serves both as cross-linker and cross-linked chain. The resulting network consists of PEG chains esterified to dense, multifunctional cross-link regions formed from long poly(acrylic acid) kinetic chains¹². Physical chain entanglements also contribute to the network (Fig. 1). It is also possible for PEGDA molecules to form intra-chain cycles and dangling ends, but these do not contribute to the network-dependent properties.

Numerous studies have attempted to develop effective strategies to control PEGDA gel properties, including increasing the ratio of PEGDA to water at the time of polymerization or decreasing the PEG chain length. These approaches result in decreased hydrogel swelling^{13–15}, increased mechanical modulus^{14,15}, and decreased effective transport of non-gaseous molecules diffusing through the gel¹³. Alterations in PEGDA concentration or size alter the kinetics of polymerization, which affects the chain length of the poly(acrylic acid) cross-linking nodes and the extent of network defects such as intra-chain cyclic structures or unreacted PEGDA. Many studies also have attempted to estimate the molecular weight between cross-links (M_c) and the mesh size (ϵ) from swelling and mechanical data^{6,13,14} using variations on the Flory-Rehner model¹⁶. These models provide estimates that generally correlate well with observed physical properties. However, by using equations derived for networks formed from long polymers with tetrafunctional junctions¹³ these analyses neglect the complex nature of the PEGDA network.

When used as a tissue engineering scaffold, the physical properties of PEGDA hydrogel scaffolds can alter the behavior of a variety of tissues including cartilage, smooth muscle, and nerve. Increasing the initial macromer concentration or decreasing PEG length results in altered gel properties that affect cell behavior directly including diminished access to proteins in the extracellular space¹³, altered extracellular matrix production and cell phenotype^{6,17}, or decreased neurite extension¹⁸. The physical properties of a tissue engineering scaffold can also limit the diffusion of nutrients and growth factors and can alter the rate of degradation of degradable PEGDA derivatives.

Without modification, PEGDA hydrogels do not support cell attachment¹⁹. Since Hern and Hubbell first reported the modification of PEGDA gels with the RGD adhesive peptide, numerous studies have developed this system as a scaffold for a variety of cell types^{3,7,19–21}. In these studies, adhesive peptides were linked to the PEGDA network via PEG-monoacrylates (PEGMA) which participate in the free radical cross-linking of the gel, resulting in non-network dangling ends (Fig 1.). This strategy has also been utilized to link growth factors, inorganic compounds and other biologically functional moieties to PEGDA gels^{22–25}. PEGMA conjugates also have been copolymerized with PEGDA to impart pH sensitivity to gel swelling or to alter the network properties for non-tissue engineering purposes^{14,26}. Despite the wealth of applications, the effects of PEGMA on PEGDA network structure and gel properties have not been studied in detail. Understanding the effect of incorporating biologically active PEGMA will facilitate the design of PEGDA

scaffolds which provide optimal biochemical and mechanical environments. In this report, we examine the effect of PEGMA on the network structure and physical properties of PEGDA hydrogels generated from macromer materials similar to those used for tissue engineering.

Materials and Methods

Materials

Poly(ethylene glycol) (PEG, M_n 6000), monomethoxy-PEG (M_n 2000 or 5000), triethylamine, acryloyl chloride, and anhydrous dichloromethane were purchased from Aldrich and used as received. Irgacure 2959 (1-[4-[2-Hydroxyethoxy]-phenyl]-2-hydroxy-2-methyl-1-propane-1-one) was purchased from Ciba Specialty Chemicals (Tarrytown, NY). Phosphate buffered saline (PBS) was prepared in distilled water and adjusted to pH 7.4. Chemical structures of relevant compounds are shown in Table 1.

Synthesis of Hydrogel Precursors

PEGDA ($M_n \approx 6000$) and PEGMA ($M_n \approx 2000$ or 5000) were prepared by conjugation of PEG-diol or monomethoxy-PEG-OH, respectively, with a 2-fold molar excess of acryloyl chloride relative to free hydroxyls in the presence of triethylamine in anhydrous dichloromethane under nitrogen overnight. The resulting products were precipitated in ice cold ether, filtered, dried in a vacuum oven at room temperature, and stored at -20°C . The resulting product was characterized by matrix assisted laser desorption/ionization time of flight mass spectroscopy (MALDI-MS) and proton NMR.

Hydrogel Formation

PEGDA-co-PEGMA hydrogels were formed at various compositions (PEGMA: 0–20 % w/w, PEGDA: 0–20% w/w, PBS: 60–95% w/w) using 0.1% (w/v) Irgacure 2959 photoinitiator. Hydrogel precursors were formed from stock solutions of PEGDA (44% w/w), PEGMA (44% w/w), PBS, and Irgacure 2959 (1% w/v in PBS). The density of PEG solutions, which is linear and independent of MW for 2–12 kDa PEG in this composition range, was used to adjust the volumes of each solution to achieve the appropriate final mass composition for each hydrogel. 100 μl of each precursor solution was dispensed into a stainless steel mold ($D = 10$ mm, $H = 1.2$ mm) and polymerized for 10.0 min at 0.4–0.5 mW/cm^2 under a 365 nm UV lamp at room temperature. Three gels were formed for each composition studied.

Properties Testing

Hydrogels were allowed to swell in excess PBS (pH 7.4) at room temperature ($22\text{--}23^\circ\text{C}$) for 4 days. A 10 mm circle was punched from the center of each swollen gel, massed, and mounted between 10 mm circular compression platens in a Dynamic Mechanical Analyzer 7e (Perkin-Elmer). The mechanical properties of each gel were evaluated in unconfined compression in a PBS bath at a rate of 50 mN/min . The shear modulus was determined from the slope of σ vs. $-(\lambda - \lambda^{-2})$ where λ is the ratio of the deformed height of the gel to the pre-deformation height¹⁴. The linear region used to determine the shear modulus was typically between 2 and 25% compressive strain. Non-linear regions of the σ vs. $-(\lambda - \lambda^{-2})$ plot for small values of $-(\lambda - \lambda^{-2})$ (during initial platen-gel contact) were excluded from the analysis. For the strongest and weakest gels, the tests were repeated after 1 day of re-equilibration with PBS at a rate of 25 mN/min to assess the effects of stress-rate on the measured modulus.

After mechanical testing, each gel was incubated with excess distilled water for an additional day to leach buffer salts and lyophilized. The mass swelling ratio, q , was determined using the following equation:

$$q = \frac{m_s}{m_p} \quad (1)$$

where m_s and m_p are the mass of the swollen gel in PBS and the mass of the polymer network, respectively.

Determination of minimal PEGDA concentration for gel formation

To investigate the properties of PEGDA-co-PEGMA gels with minimal PEGDA content, hydrogels with PEGMA (20% w/w) were polymerized with a range of low concentrations of PEGDA (0.1–5%, w/w) as described above. The mass swelling ratio was determined gravimetrically.

Hydrogel Network Composition

After testing gel properties, selected networks were degraded in 1 N sodium hydroxide, dialyzed (500 MWCO), and lyophilized yielding samples containing monomethoxy-PEG (mPEG5k) and diol-PEG (PEG6k) corresponding to polymerized PEGMA and PEGDA, respectively. Relative PEGMA vs. PEGDA incorporation was determined using the relative size of the mPEG5k vs. PEG6k distributions on MALDI-MS spectra of the degraded gel samples. Spectra of samples with known mPEG5k/PEG6k compositions were used to form a linear calibration curve ($r^2 > 0.99$).

Analysis of Hydrogel Network Structure

Since the hydrogels studied here were formed in solution, the method of Bray and Merrill was adapted to calculate v_e , the effective cross-linked chains per gram of polymer, using the following equation²⁷:

$$v_e = \frac{\ln(1 - v_{p,s}) + v_{p,s} + \chi v_{p,s}^2}{-\bar{V}_s \rho_p v_{p,r} \left[\left(\frac{v_{p,s}}{v_{p,r}} \right)^{\frac{1}{3}} - \frac{2}{F} \left(\frac{v_{p,s}}{v_{p,r}} \right) \right]} \quad (2)$$

where:

$$v_{p,s} = \frac{1}{\frac{\rho_p}{\rho_s}(q - 1) + 1} \quad (3)$$

$$v_{p,r} = \frac{1}{\frac{\rho_p}{\rho_s} \left(\frac{1}{f} - 1 \right) + 1} \quad (4)$$

and χ is the Flory-Huggins interaction parameter for PEG and water (0.426)⁴, $\bar{V}_s$ is the molar volume of the solvent, water (18.0 cm³ mol⁻¹), ρ_p is the bulk density of PEG (1.18 g ml⁻¹)¹⁴, F is the junction functionality, ρ_s is the density of PBS (1.01 g ml⁻¹), and f is the mass fraction of polymer in solution at the time of cross-linking.

The PEGMA-co-PEGDA hydrogels contain various compositions of cross-linking (PEGDA) and non-cross-linking elements (PEGMA). To characterize the nature of these networks, the number of effective cross-links was normalized to the number of cross-link forming PEGDA molecules in each gel at the time of cross-linking to give a new quantity, v_e^* defined as:

$$v_e^* = v_e \cdot \frac{f}{f_{PEGDA}} \cdot MW_{PEGDA} \quad (5)$$

where f_{PEGDA} is the mass fraction of PEGDA at the time of polymerization and MW_{PEGDA} is the number averaged molecular weight of the PEGDA. In a perfect network with no entanglements, v_e^* is equal to 1 since all of the PEGDA chains participate in the network. Because F is not explicitly determined by the chemical structure of the gels, v_e^* was fit to swelling data for $4 < F \leq 10^6$.

The network structure was also calculated independently from mechanical data using an affine model of rubber elasticity for isovolumetric compression¹⁶. After normalizing to the number of PEGDA molecules, the relationship between the shear modulus and v_e^* was:

$$v_e^* = \frac{G \cdot MW_{PEGDA}}{RT v_p (\rho_p \cdot 10^6) \left(\frac{f_{PEGDA}}{f} \right)} \quad (6)$$

where G is the shear modulus (in N m^{-2}), R is the gas constant ($8.413 \text{ J K}^{-1} \text{ mol}^{-1}$), and T is the absolute temperature (K), and the units of ρ_p and MW_{PEGDA} are g cm^{-3} and g mol^{-1} , respectively. The 10^6 term converts density from g cm^{-3} to g m^{-3} .

Photopolymerization of PEGMA

Poly(PEGMA) homopolymers were prepared by polymerizing various concentrations of PEGMA ($M_n = 2 \text{ kDa}$, 10–50 % w/w) as described for the PEGDA-co-PEGMA hydrogels. After polymerization the viscous liquids were transferred from the mold and lyophilized.

Gel Permeation Chromatography

Lyophilized poly(PEGMA) was reconstituted at 5 mg/ml in deionized water and filtered through a $0.22 \mu\text{m}$ filter. GPC was performed using 3 HEMA BIO 100 (Tessek SEPARO) size exclusion columns in series with a flow rate of 1 ml/min and 100 μl sample volumes. Light scattering and refractive index measurements were acquired using a Waters 2690 separations module with a Waters 410 refractive index detector and a Wyatt MiniDAWN light scattering detector. The software package associated with the system (ASTRA, version 4.0) was used to calculate the molecular weight (M_n and M_w) of the PEGMA homopolymers using a dn/dc for PEG of 0.1360. The extent of polymerization was determined by dividing M_n of the homopolymer by the M_n of the PEGMA (2 kDa). 2 kDa PEGMA was substituted for 5 kDa PEGMA to bring the molecular weight of the resulting branched polymer into a range suitable for our separation columns.

Statistics

Data are represented as mean $\pm$ standard deviation of at least 3 samples. Statistical analysis was performed using student's t-tests for single comparisons. P-values < 0.05 were considered significant.

Results

PEGDA, PEGMA Synthesis

MALDI-MS spectra of PEGDA and PEGMA showed a shift to higher molecular weight of roughly 108 and 54 respectively corresponding to the conjugation of two or one acrylate groups. The PEGMA spectra showed two families of peaks consistent with the presence of acrylated and free hydroxyl PEGs and did not show evidence for the presence of any diacrylated PEG. Proton NMR demonstrated approximately 75% and 95% acrylation for 2 kDa PEGMA and 5 kDa PEGMA, respectively.

Hydrogel Network Composition

The recovered mass of the copolymer hydrogel networks increased proportionally with PEG concentration in the initial macromer solution (PEGMA+PEGDA) with no dependence on the PEGMA:PEGDA ratio, suggesting that the incorporation of PEGMA and PEGDA approximately mirrored the composition of the initial macromer solution. The overall conversion increased from $78 \pm 5\%$ toward 100% as the PEG concentration increased from 5 to 40% wt/wt. MALDI-MS analysis of selected 10%PEGDA/10%PEGMA and 10%PEGDA/20%PEGMA gels revealed estimated fractions of PEGMA in the final polymer network of 0.57 ± 0.01 and 0.71 ± 0.004 respectively.

Hydrogel Swelling

For fixed concentrations of PEG (PEGDA+PEGMA), increasing the mol fraction of PEGDA in the macromer feed resulted in a decreased swelling ratio, as expected (Fig 2A). The swelling ratio of PEGDA-co-PEGMA hydrogels decreased substantially with increasing PEGDA composition (Fig. 3A). The substitution of polymerization-inactive PBS with polymerization-active PEGMA decreased the swelling ratio by up to $42 \pm 1.6\%$. The effect was most pronounced for gels with low PEGDA compositions. PEGDA gels used for tissue engineering applications are often supplemented with biologically active PEGMA. The addition of PEGMA (shown in units of mM) to gels with fixed PEGDA composition resulted in a decreased swelling ratio only when the concentration of PEGMA was high relative to the concentrations used to promote cell attachment (< 7 mM) and the PEGDA concentration was low (Fig. 3B) 3.19 ± 28 .

As the concentration of cross-linking PEGDA was decreased in PEGDA-co-PEGMA gels containing fixed PEGMA (5 kDa, 20% w/w), the swelling ratio of the resulting gels increased asymptotically (Fig. 4). A minimum concentration of 1% PEGDA (1:20 PEGDA:PEGMA) was necessary to induce gel formation. Controls formed from 0% PEGDA/20% PEGMA did not form gel networks.

Hydrogel Mechanical Properties

The shear modulus of the hydrogels was calculated from the slope of σ vs. $-(\lambda - \lambda^{-2})$. The value of r^2 was greater than 0.988 for all regression fits. For fixed concentrations of PEG (PEGDA+PEGMA), increasing the mol fraction of PEGDA in the macromer feed resulted in an increased shear modulus, as expected (Fig 2B). The shear modulus increased with increasing PEGDA composition (Fig 5). The addition of PEGMA generally resulted in an increase in shear modulus that was proportionately similar, relative to the 0% PEGMA, for each PEGDA concentration studied (up to $167 \pm 29.3\%$).

After one day of re-equilibration in PBS, a reduction in the rate of stress application from 50 mN/min to 25 mN/min resulted in a decrease in the apparent modulus from 4.0 ± 0.6 kPa to 3.4 ± 0.3 kPa (14.7%, $p=0.047$) for the 5% PEGDA/0% PEGMA gels and from 104.2 ± 4.0 kPa to 102.6 ± 18.5 kPa (1.6 %, $p=0.861$) for the 20% PEGDA/20% PEGMA gels.

Hydrogel Network Structure

The effective number of cross-linked chains per PEGDA molecule, ν_e^* , calculated with Eq. 5, was used to estimate the network structure from swelling data. A sensitivity analysis on the effect of the unknown parameter F on ν_e^* showed that ν_e^* decreased asymptotically with increasing F , approaching a value 20–40% less than the $F=4$ calculation for PEGDA gels containing 0% PEGMA (Fig. 6). The results for hydrogels containing 5–20% (w/w) PEGMA followed a similar trend (not shown).

To assess the network structure of PEGDA-co-PEGMA gels, ν_e^* was calculated from both swelling and mechanical data using $F=4$ (Figs. 7 and 8, respectively). The value of ν_e^* was directly proportional to the PEGDA composition (Figs. 7A and 8A). The addition of PEGMA increased ν_e^* while having a minimal impact on the slope of the ν_e^* vs. PEGDA relationship. The addition of PEGMA to gels with fixed PEGDA composition resulted in an increase in ν_e^* (Figs. 7B and 8B). ν_e^* estimated from mechanical data, which did not depend on junction functionality, was generally well below the range of possible ν_e^* values calculated from swelling data, but followed the same trends.

The value of ν_e^* gives an estimate of the type of cross-linking occurring in these network structures. For most of the gels ν_e^* was greater than 1 when assessed using both swelling and mechanical data (even in the limiting case $F \rightarrow \infty$), and for many of the gels with high compositions of PEGMA or PEGDA, ν_e^* was greater than 2, suggesting strongly that PEGDA entanglements play a critical role in the network structures of these materials.

Monoacrylated PEG Homopolymerization

The degree of polymerization of PEGMA macromers polymerized without PEGDA under the same conditions as the gels was determined using GPC with absolute molecular weight determined by light scattering and refractometry data. The degree of polymerization increased monotonically with PEGMA concentration (Fig. 9).

Discussion

PEG di(meth)acrylate hydrogels copolymerized with PEGMA have been employed as tissue engineering scaffolds for many applications including bone, cartilage, nerve, and vascular tissues^{3,17,18,21}. The goal of this work was to examine the effects of this copolymerization on the properties and network structure of the resulting hydrogels.

We focused our investigation on a single combination of PEGDA and PEGMA macromers. 6 kDa PEGDA is typical of the range commonly used for tissue engineering (3.4 kDa–8 kDa) ^{3,20,29,30} and 5 kDa PEGMA is similar in size to peptide-PEG-acrylate (for example, GRGDSP-PEGMA has a MW of ~4200). A neutral methyl-PEG terminus was employed to avoid confounding effects from specific peptide-PEGMA-solvent interactions which can alter gel properties²⁶.

The PEGDA composition at the time of cross-linking played a dominant role in determining the network structure (Figs. 3A and 5A). However, the substitution of PEGMA for PBS in the prepolymerization macromer solution resulted in a decreased swelling ratio and an increased shear modulus, suggesting that the additional PEGMA increased the network cross-linking. Since the PEGMA does not contribute to the elastic component of the network, this result was unexpected.

A new parameter ν_e^* , the number of effective cross-links per PEGDA molecule, was defined to better describe the network of PEGDA-co-PEGMA gels. Previous reports characterized PEGDA networks by calculating M_c ^{6,13,14,29} using variations of the Bray-Merrill

model²⁷, which was derived for networks formed from long polymer chains cross-linked with tetrafunctional linkages in solution. Given that (1) PEGDA networks are formed from short polymer chains cross-linked by polymerization of junctions of unspecified functionality (Fig. 1), (2) there is no appropriate M_n to characterize the long polymer chains from which the network is to be sub-divided, and (3) the Bray-Merrill model does not account for the PEGMA component of the copolymer gels, v_e^* better characterizes PEGDA and PEGDA-co-PEGMA systems. However, like original the Bray-Merrill model, our approach relies upon a statistical thermodynamic based derivation of the entropies of solvation and distension. Thus, observations that the cross-linking nodes may form discrete microdomains within the gels¹² suggest their may be limitations to this approach.

Because the value of F was unknown in our experiments, we conducted a sensitivity analysis for this parameter, which indicated that the value of F had minimal impact on the magnitude and trends for v_e^* , even if F was allowed to vary as a function of PEGDA composition ($F=4$ for 5% PEGDA to $F \rightarrow \infty$ for 20% PEGDA). Since the trends were unaltered by F , we performed subsequent calculations using $F=4$. Although this over estimates v_e^* for these gels, this value has been used implicitly to model PEGDA hydrogels by others¹³ and serves as an upper limit on the calculated value of v_e^* .

This analysis of the data supports the conclusion that the addition of PEGMA to PEGDA polymerizations results in increased cross-linking of the network. Others have reported that replacement of buffer with PEGMA did not affect the properties of gel networks made with 743 Da PEGDA and 460 Da PEGMA¹⁴. For similar mass compositions, these smaller molecular weight materials resulted in gels with a cross-link density (effective chains per volume of polymer) 1–2 orders of magnitude higher than those observed in this study. This difference suggests that gels formed in solution with lower concentrations of reactive acrylate groups are more susceptible to the increased cross-linking via the addition of PEGMA, supporting a polymerization kinetics based explanation for the observed effects.

Calculations of v_e^* from swelling data (for the limiting case of $F \rightarrow \infty$) and mechanical data, predicted a greater number of cross-link units in the gels than the number of PEGDA units for many of the compositions studied (Figs. 7 and 8). This result is highly suggestive that chain entanglements play a critical role in the network structure of these materials. Entanglements, if present, would represent a second set of cross-links in the network with a junction functionality of ~ 4 and a second distribution of chain lengths which are subdivisions of the PEGDA chains, suggesting that a simple statistical thermodynamic model is insufficient to fully describe the network structure of these PEGDA gels and may explain the discrepancy between mechanical and swelling data. The potential for a bimodal distribution of chain lengths and junction functionalities in the entangled PEGDA networks further suggests that M_c derived from these network models (which for PEGDA-co-PEGMA gels is most appropriately calculated by dividing the mass of cross-linking polymer by the number of cross-linking chains in moles, equivalent to dividing M_n of the PEGDA by v_e^*) does not adequately represent the physical dimension inside these copolymerized gels and may not correlate well with actual gel mesh size or transport properties, as is the case with traditionally cross-linked networks³¹.

Although the polymerization kinetics within these diacrylate networks is complex^{32,33}, a simplified model of the free radical polymerization suggests that the kinetic chain length of the resulting poly(acrylic acid) nodes should be roughly proportional to initial concentration of acrylate groups, which is increased with increasing PEGMA³⁴. In the absence of gelation, we observed a direct relationship between the initial PEGMA concentration and the resulting polymer chain length, with a degree of polymerization on the order of 100's of repeat units. These results are consistent with Burdick *et al.* who reported chain lengths on

the order of 1000's for more highly cross-linked polyanhydride diacrylate networks¹¹. While the degree of polymerization seen in this experiment cannot be related to the polyacrylic acid nodes within the PEGDA hydrogels directly, these results suggest that the functionality of the nodes could be quite high. An increased kinetic chain length of the resulting nodes would be expected to increase the junction functionality F , although this increase is not 1:1 since both PEGDA and non-network forming PEGMA are incorporated into the chain. The PEGMA-extended poly(acrylic acid) nodes, if present, would also fix additional chain entanglements in the network, as suggested by the analysis of ν_e^* . These results also suggest that copolymerization of other acrylate functionalized materials like poly(vinyl alcohol), hyaluronic acid, or fibrinogen^{8–10} with additional monofunctional material likewise may enhance the cross-linking of these systems.

In tissue engineering applications, the concentration of PEGMA is much lower than PEGDA, typically 7 mM or less^{3·19·28}. The effects of PEGMA demonstrated in this study were most apparent as the concentration approached or exceeded the concentration of PEGDA (Figs 3B· 5B· 7B, and 8B). These results suggest that the low concentration of PEGMA (conjugated with a high affinity ligand like RGD) currently used for tissue engineering applications has a minimal effect on the gel's network. However, several groups are beginning to report the incorporation of multiple peptides or growth factors^{30·35}. Others have reported the incorporation of inorganic groups or drugs to enhance the biological activity of these scaffolds^{23·25}. A tissue engineering scaffold that can incorporate a wide variety of biologically active factors at high concentration may more effectively mimic the natural extracellular space or provide more potent cues for cell behavior. Our results suggest that the formation of robust hydrogel scaffolds with a high concentration of biologically active moieties is feasible using a copolymerization approach.

Conclusions

The addition of PEGMA to photopolymerized PEGDA hydrogels similar to those used in tissue engineering applications enhanced the network cross-linking, as evidenced by a decreased swelling ratio, increased mechanical modulus, and an increase in the calculated effective chains per cross-linking PEGDA molecule, ν_e^* . Further analysis of the network indicated that this enhanced cross-linking occurred via the formation of entanglements which were fixed by extended poly(acrylic acid) cross-linking nodes in the networks. The results suggest that PEGMA-co-PEGDA gels can be formed with higher concentrations of PEGMA-tethered ligands than previously reported allowing the formation of scaffolds with a rich diversity of biological functionalities without sacrificing the integrity of the gel network.

Acknowledgments

The project described was support by Grant Number 5R01EB002067 for the National Institute of Biomedical Imaging and Bioengineering. The authors would like to acknowledge the facilities provided by the Center for Cardiovascular Biomaterials. JAB was also supported by NIH T32GM07250 and American Heart Association Predoctoral Fellowship 0715422B.

References

1. Shin H, Jo S, Mikos AG. Modulation of marrow stromal osteoblast adhesion on biomimetic oligo[poly(ethylene glycol) fumarate] hydrogels modified with Arg-Gly-Asp peptides and a poly(ethyleneglycol) spacer. *J Biomed Mater Res*. 2002; 61(2):169–79. [PubMed: 12061329]
2. Lutolf MP, Lauer-Fields JL, Schmoekel HG, Metters AT, Weber FE, Fields GB, Hubbell JA. Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration:

- engineering cell-invasion characteristics. *Proc Natl Acad Sci U S A*. 2003; 100(9):5413–8. [PubMed: 12686696]
3. Burdick JA, Anseth KS. Photoencapsulation of osteoblasts in injectable RGD-modified PEG hydrogels for bone tissue engineering. *Biomaterials*. 2002; 23(22):4315–23. [PubMed: 12219821]
 4. Merrill EW, Dennison KA, Sung C. Partitioning and diffusion of solutes in hydrogels of poly(ethylene oxide). *Biomaterials*. 1993; 14(15):1117–26. [PubMed: 8130315]
 5. Hahn MS, Taite LJ, Moon JJ, Rowland MC, Ruffino KA, West JL. Photolithographic patterning of polyethylene glycol hydrogels. *Biomaterials*. 2006; 27(12):2519–24. [PubMed: 16375965]
 6. Bryant SJ, Anseth KS. Hydrogel properties influence ECM production by chondrocytes photoencapsulated in poly(ethylene glycol) hydrogels. *J Biomed Mater Res*. 2002; 59(1):63–72. [PubMed: 11745538]
 7. Mann BK, Gobin AS, Tsai AT, Schmedlen RH, West JL. Smooth muscle cell growth in photopolymerized hydrogels with cell adhesive and proteolytically degradable domains: synthetic ECM analogs for tissue engineering. *Biomaterials*. 2001; 22(22):3045–51. [PubMed: 11575479]
 8. Masters KS, Shah DN, Leinwand LA, Anseth KS. Crosslinked hyaluronan scaffolds as a biologically active carrier for valvular interstitial cells. *Biomaterials*. 2005; 26(15):2517–25. [PubMed: 15585254]
 9. Almany L, Seliktar D. Biosynthetic hydrogel scaffolds made from fibrinogen and polyethylene glycol for 3D cell cultures. *Biomaterials*. 2005; 26(15):2467–77. [PubMed: 15585249]
 10. Martens P, Anseth KS. Characterization of hydrogels formed from acrylate modified poly(vinyl alcohol) macromers. *Polymer*. 2000; 41(21):7715–7722.
 11. Burdick JA, Lovestead TM, Anseth KS. Kinetic chain lengths in highly cross-linked networks formed by the photoinitiated polymerization of divinyl monomers: A gel permeation chromatography investigation. *Biomacromolecules*. 2003; 4(1):149–156. [PubMed: 12523860]
 12. Lin-Gibson S, Jones RL, Washburn NR, Horkay F. Structure-property relationships of photopolymerizable poly(ethylene glycol) dimethacrylate hydrogels. *Macromolecules*. 2005; 38(7):2897–2902.
 13. Cruise GM, Scharp DS, Hubbell JA. Characterization of permeability and network structure of interfacially photopolymerized poly(ethylene glycol) diacrylate hydrogels. *Biomaterials*. 1998; 19(14):1287–94. [PubMed: 9720892]
 14. Lin HQ, Kai T, Freeman BD, Kalakkunnath S, Kalika DS. The effect of cross-linking on gas permeability in cross-linked poly(ethylene glycol diacrylate). *Macromolecules*. 2005; 38(20):8381–8393.
 15. Lin-Gibson S, Bencherif S, Cooper JA, Wetzel SJ, Antonucci JM, Vogel BM, Horkay F, Washburn NR. Synthesis and characterization of PEG dimethacrylates and their hydrogels. *Biomacromolecules*. 2004; 5(4):1280–7. [PubMed: 15244441]
 16. Flory, PJ. Principles of polymer chemistry. Ithaca: Cornell University Press; 1953.
 17. Peyton SR, Raub CB, Keschrumrus VP, Putnam AJ. The use of poly(ethylene glycol) hydrogels to investigate the impact of ECM chemistry and mechanics on smooth muscle cells. *Biomaterials*. 2006; 27(28):4881–93. [PubMed: 16762407]
 18. Gunn JW, Turner SD, Mann BK. Adhesive and mechanical properties of hydrogels influence neurite extension. *J Biomed Mater Res A*. 2005; 72(1):91–7. [PubMed: 15536643]
 19. Hern DL, Hubbell JA. Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing. *J Biomed Mater Res*. 1998; 39(2):266–76. [PubMed: 9457557]
 20. Nuttelman CR, Tripodi MC, Anseth KS. In vitro osteogenic differentiation of human mesenchymal stem cells photoencapsulated in PEG hydrogels. *J Biomed Mater Res A*. 2004; 68(4):773–82. [PubMed: 14986332]
 21. Hwang NS, Varghese S, Zhang Z, Elisseeff J. Chondrogenic differentiation of human embryonic stem cell-derived cells in arginine-glycine-aspartate-modified hydrogels. *Tissue Eng*. 2006; 12(9):2695–706. [PubMed: 16995803]
 22. DeLong SA, Moon JJ, West JL. Covalently immobilized gradients of bFGF on hydrogel scaffolds for directed cell migration. *Biomaterials*. 2005; 26(16):3227–3234. [PubMed: 15603817]

23. Nuttelman CR, Benoit DS, Tripodi MC, Anseth KS. The effect of ethylene glycol methacrylate phosphate in PEG hydrogels on mineralization and viability of encapsulated hMSCs. *Biomaterials*. 2006; 27(8):1377–86. [PubMed: 16139351]
24. Cushing MC, Liao JT, Jaeggli MP, Anseth KS. Material-based regulation of the myofibroblast phenotype. *Biomaterials*. 2007; 28(23):3378–87. [PubMed: 17475322]
25. Benoit DS, Nuttelman CR, Collins SD, Anseth KS. Synthesis and characterization of a fluvastatin-releasing hydrogel delivery system to modulate hMSC differentiation and function for bone regeneration. *Biomaterials*. 2006; 27(36):6102–10. [PubMed: 16860387]
26. Kim JH, Kim JG, Kim D, Kim YH. Preparation and properties of PEG hydrogel from PEG macromonomer with sulfonate end group. *Journal of Applied Polymer Science*. 2005; 96(1):56–61.
27. Bray JC, Merrill EW. Poly(vinyl alcohol) hydrogels. Formation by electron beam irradiation of aqueous solutions and subsequent crystallization. *Journal of Applied Polymer Science*. 1973; 17:3779–3794.
28. Mann BK, West JL. Cell adhesion peptides alter smooth muscle cell adhesion, proliferation, migration, and matrix protein synthesis on modified surfaces and in polymer scaffolds. *J Biomed Mater Res*. 2002; 60(1):86–93. [PubMed: 11835163]
29. Buxton AN, Zhu J, Marchant R, West JL, Yoo JU, Johnstone B. Design and characterization of poly(ethylene glycol) photopolymerizable semi-interpenetrating networks for chondrogenesis of human mesenchymal stem cells. *Tissue Eng*. 2007; 13(10):2549–60. [PubMed: 17655489]
30. Mann BK, Schmedlen RH, West JL. Tethered-TGF-beta increases extracellular matrix production of vascular smooth muscle cells. *Biomaterials*. 2001; 22(5):439–44. [PubMed: 11214754]
31. Peppas, NA. *Hydrogels in medicine and pharmacy*. Boca Raton, Fla: CRC Press; 1986.
32. Anseth KS, Kline LM, Walker TA, Anderson KJ, Bowman CN. Reaction-Kinetics and Volume Relaxation during Polymerizations of Multiethylene Glycol Dimethacrylates. *Macromolecules*. 1995; 28(7):2491–2499.
33. Ward JH, Furman K, Peppas NA. Effect of monomer type and dangling end size on polymer network synthesis. *Journal of Applied Polymer Science*. 2003; 89(13):3506–3519.
34. Odian, GG. *Principles of polymerization*. Vol. xxiv. Hoboken, N.J: Wiley-Interscience; 2004. p. 812
35. Weber LM, Hayda KN, Haskins K, Anseth KS. The effects of cell-matrix interactions on encapsulated beta-cell function within hydrogels functionalized with matrix-derived adhesive peptides. *Biomaterials*. 2007; 28(19):3004–11. [PubMed: 17391752]

Figure 1.

Schematic representation of the PEGDA network showing (a) cross-linked PEG chains and network defects including (b) unreacted acrylate moieties, (c) PEG cycles, (d) chain entanglements, and (e) PEG tethered biological moieties. Shaded arrow shapes represent reacted acrylates in a poly(acrylic acid) cross-linking node, unshaded arrow shapes represent unreacted acrylate, dark lines represent PEG chains esterified to the acrylic acid, and the shaded hexagon represents a biological moiety. For clarity, short poly(acrylic acid) chains are shown, but in actual gels these chain lengths may be much longer.

Figure 2.

Mass swelling ratios (A) and shear moduli (B) of PEGDA-co-PEGMA hydrogels shown as a function of the mole fraction of PEGDA in the macromer feed (mol PEGDA/[mol PEGDA + mol PEGMA]). Each curve shows a fixed overall concentration of PEG in PBS (% PEGDA + % PEGMA, w/w) at the time of polymerization.

Figure 3.

Mass swelling ratios of PEGDA-co-PEGMA hydrogels shown as a function of (A) the concentration of PEGDA (% wt/wt) at the time of cross-linking supplemented with various concentrations of PEGMA (% wt/wt), or (B) the molar concentration of PEGMA (mM) in the solution at the time of cross-linking for various PEGDA compositions (% wt/wt). PEGMA concentration was expressed in mM to facilitate comparison with the tissue engineering literature.

Figure 4.

Mass swelling ratio of PEGDA-co-PEGMA hydrogels formed from 20% PEGMA (wt/wt) and decreasing amounts of PEGDA. The shaded area indicates PEGDA compositions which did not support the formation of networks. Hydrogels formed from PEGDA alone are shown for comparison.

Figure 5.

Shear moduli of PEGDA-co-PEGMA hydrogels determined using an affine network model shown as a function of (A) concentrations of PEGDA (% wt/wt) at the time of cross-linking supplemented with various concentrations of PEGMA (% wt/wt) or (B) the molar concentration of PEGMA (mM) in the solution at the time of cross-linking for various PEGDA concentrations (% wt/wt). PEGMA concentration was expressed in mM to facilitate comparison with the tissue engineering literature.

Figure 6. v_e^* , the number of effective cross-links per PEGDA molecule for PEGDA (0% PEGMA) hydrogels calculated from swelling data using the equation 5 for various values of F , the junction functionality. Calculations performed for $100 < F \leq 10^6$ are not shown for clarity but were within 1.7% of the $F = 100$ values. v_e^* calculated independently from mechanical data is also shown for reference.

Figure 7. ν_e^* , the number of effective cross-links per PEGDA molecule for various PEGDA-co-PEGMA hydrogels calculated from swelling data shown as a function of (A) the concentration of PEGDA (% wt/wt) at the time of cross-linking supplemented with various concentrations of PEGMA (% wt/wt) or (B) the molar concentration of PEGMA (mM) in the solution at the time of cross-linking for various PEGDA concentrations (% wt/wt). ν_e^* was estimated using equation 5 with a junction functionality (F) of 4.

Figure 8. ν_e^* , the number of effective cross-links per PEGDA molecule for various PEGDA-co-PEGMA hydrogels calculated from mechanical data shown as a function of (A) the concentration of PEGDA (% wt/wt) at the time of cross-linking supplemented with various concentrations of PEGMA (% wt/wt) or (B) the molar concentration of PEGMA (mM) in the solution at the time of cross-linking for various PEGDA concentrations (% wt/wt). ν_e^* was estimated using equation 6.

Figure 9. Number averaged degree of polymerization of PEGMA (2 kDa) homopolymer formed under the same conditions as the hydrogels studied here as determined by gel permeation chromatography.

Table 1

Chemical Structure of PEGDA and PEGMA

Chemical	Structure
poly(ethylene glycol) diacrylate (PEGDA, MW = 6 kD)	$\text{CH}_2=\text{CH}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}\left(\text{O}-\text{CH}_2-\text{CH}_2\right)_{133}\text{O}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{CH}=\text{CH}_2$
poly(ethylene glycol) monoacrylate methyl ether (PEGMA, MW = 5 kD)	$\text{CH}_2=\text{CH}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}\left(\text{O}-\text{CH}_2-\text{CH}_2\right)_{111}\text{O}-\text{CH}_3$