Journal of Applied Polymer Science

Designing crosslinked hyaluronic acid hydrogels with tunable mechanical properties for biomedical applications

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ABSTRACT: This study develops a simple copolymerization/crosslinking technique to control the swelling and mechanical properties of hyaluronic acid-based hydrogels. Because of the widespread acceptance of poly(ethylene glycol) in biomedical applications, functionalized oligomers of ethylene glycol (EG) were used as comonomers to crosslink methacrylated hyaluronic acid (MHA). The swelling degree, shear and elastic moduli, and fracture properties (stress and strain) of the gels were investigated as a function of the crosslinking oligomer length and reactive group(s). It was hypothesized that acrylated oligomers would increase the crosslink density of the gels through formation of kinetic chains by reducing the steric hindrances that otherwise may limit efficient crosslinking of hyaluronic acid into gels. Specifically, after crosslinking 13 wt % MHA (47% degree of methacrylation) with 0.06 mol % of $(EG)_n$-diacrylate, the swelling ratio of the MHA gel decreased from 27 to 15 *g*/*g* and the shear modulus increased from 140 to 270 kPa as *n* increased from 1 to 13 units. The length and functionality (i.e., acrylate vs. methacrylate) of the oligomer controlled the crosslink density of the gels. The significant changes in the gel properties obtained with the addition of low levels of the PEG comonomer show that this method allows precise tuning of the physical properties of hyaluronic acid (HA) gels to achieve desired target values for biomedical applications. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42009.

KEYWORDS: biopolymers; copolymers; crosslinking; gels; photopolymerization

Received 7 September 2014; accepted 18 January 2015
DOI: 10.1002/app.42009

INTRODUCTION

Due to excellent biological and viscoelastic properties of hyaluronic acid (HA), hydrogels made from HA have gotten considerable attention as biomaterials. Tissue engineering applications make use of HA-derived hydrogels to form biocompatible and degradable scaffolds.[1–3] In these applications, precise control of water content and mechanical properties has been shown necessary to direct the proliferation and differentiation of encapsulated cells in the desired manner.[4] Most commonly, these properties are adjusted by varying the concentration of the HA pre-gel solution, the degree of modification of HA, or the molecular weight of the macromer.[5,6] However, changes in the HA concentration or extent of modification can affect cellular behavior in ways unrelated to the moduli or water content of the gels. Furthermore, it can be difficult in practice to achieve batch-to-batch reproducibility in the degree of substitution and molecular weight distribution of HA macromers necessary to meet the target properties. By improving the mechanistic understanding of the HA gel formation during photopolymerization, this study presents a copolymerization strategy that can be used to tune the water content and moduli to desired values for a particular application. This concept has been previously tested in photopolymerized methacrylated chondroitin sulfate (MCS) hydrogels.[7] It was then hypothesized that this technique is applicable to other methacrylated polysaccharide gels. Here, due to the importance of hyaluronic acid, HA has been selected to confirm this hypothesis to create tunable three-dimensional scaffolds.

HA is a linear nonsulfated glycosaminoglycan (GAG) abundantly found in the mammalian extracellular matrix (ECM). In the ECM, aggrecan is made of aggregated proteoglycans bound to a backbone of hyaluronic acid.[6,8] HA has been recognized for multiple roles within the mammalian tissue such as water homeostasis, protein binding within the ECM and the cell cytosol,[9] and steric exclusion of other molecules. HA affects cell proliferation, differentiation, and motility by controlling the ECM elasticity and stiffness.[10–12] HA has been used in biomedical applications due to the wide range of obtainable molecular weights, diverse modification chemistry, possibility of enzymatic remodeling in cell culture, and nonadhesive

nature toward the cell.[11] Excellent articles on the biological roles of HA, its modification chemistry and the biomedical applications of this polymer and its hydrogels provide further details for review.[9,13–19]

Photoinitiated crosslinking of methacrylated HA (MHA) was selected as the method in this study due to fast reaction kinetics, *in vitro* and *in situ* applicability, and biocompatibility of the method for tissue engineering applications.[17,20,21] It has been shown that photocrosslinking of MHA with poly(ethylene glycol)-acrylate (PEG-acrylate) can improve the crosslink density[22] and modulate bioactivity of the gels.[23] However, the role of PEG length and acrylate groups in reaction mechanism has not been clear. The presence of residual vinyl groups after photopolymerization was shown in our previous study on crosslinked methacrylated chondroitin sulfate (MCS) gels.[7] Unreacted vinyl groups are indicators of polymerization inefficiencies, possibly a result of steric hindrances and structural conformation of chondroitin sulfate (CS). As CS and HA have similar structures with comparable conformation and persistence length values,[24] we speculated that the low crosslink densities of MHA gels (previously reported[22]) are due to similar reasons. To overcome steric limitations of macromers in solution and to increase the crosslink density of gels, oligomers of ethylene glycol diacrylate (OEGDAs) are introduced as crosslinkers to the system. The length and functional group(s) of oligomers would be used as levers to control the properties of crosslinked gels. The swelling and moduli of MHA gels can be tuned to desired values by varying length and functional groups of oligomers.

EXPERIMENTAL

Materials

Sodium hyaluronate (41 kDa–65 kDa, research grade) was purchased from Lifecore Biomedical (Chaska, MN). Ethylene glycol diacrylate ($(EG)_1DA$) (> 90%), di(ethylene glycol) diacrylate ($(EG)_2DA$) (> 75%), tetra(ethylene glycol) diacrylate ($(EG)_4DA$) (technical grade), poly(ethylene glycol) diacrylate ($(EG)_{13}DA$) (M_n 700), tetra(ethylene glycol) dimethacrylate ($(EG)_4DMA$) (> 90%), triethylamine (> 99.5%), tetrabutylammonium bromide (> 98%), and glycidyl methacrylate (GMA) were obtained from Sigma-Aldrich (St. Louis, MO) and used as received. The photoinitiator Irgacure 2959 (I-2959) was acquired from Ciba (Basel, Switzerland). Mono-functional poly(ethylene glycol) methyl ether methacrylate ($(EG)_9MEMA$) (M_n 500) and poly(ethylene glycol) methyl ether acrylate ($(EG)_9MEA$) (M_n 480) were also purchased from Sigma-Aldrich (St. Louis, MO) and used as received.

Methacrylation of Sodium Hyaluronate

Methacrylation of sodium hyaluronate followed a procedure initially described by Leach *et al.*[22] In summary, 2.0 *g* sodium hyaluronate was dissolved in 200 mL deionized water: acetone mixture (50 : 50 ratio) at room temperature. 4.4 mL triethylamine (catalyst), 4.4 mL glycidyl methacrylate, and 4.4 *g* tetrabutylammonium bromide (phase transfer catalyst) were mixed separately and were then added slowly to the macromer solution. Magnetic stirring was used to ensure homogeneous mixing of reactants in the solution. The reaction was carried out for 24 hours under the fume hood at room temperature. After 24 hours, glycidyl methacrylate-modified HA (MHA) was precipitated using 20-fold volume of acetone. After completely drying the precipitate to remove traces of volatile organic compounds, the precipitate was redissolved in deionized water. After freezing the solution at −20°C, the functionalized macromer was lyophilized at 0.03 mBar and −50°C using a 4.5 L Freezone® Labconco (Kansas City, MO) lyophilizer to dry.

Calculation of Degree of Methacrylation of HA

The precursor and functionalized macromer were characterized using ^{1}H-NMR (Bruker, 500 MHz). The degree of methacrylation of HA was calculated from the relative peak area of methacryloyl proton shifts (peaks at 5.33, 5.64, 5.74, and 6.18 ppm) to HAs methyl protons (1.95 ppm). The degree of methacrylation was calculated as 47 mol % after 1 day of reaction.

Hydrogel Preparation

Hydrogels were prepared from aqueous MHA solutions. The concentration of MHA in the gel was kept constant at 13 wt % in all formulations. For MHA gels copolymerized with OEGDA or OEGDMA, the concentration of the comonomer was fixed at 0.06 mol % (total number of moles was the summation of crosslinker, MHA disaccharides, and water moles). To keep the molar concentration of acrylate/methacrylate groups constant (as the molecular weight of the crosslinker varied), the crosslinker mass fraction was changed from 0.48 to 2.0 wt %. To study the crosslinker functional group reactivity (acrylate vs. methacrylate) effect on crosslinking efficiency, MHA gels with equimolar amounts of $(EG)_4DA$ and $(EG)_4DMA$ as crosslinker were prepared. Monofunctional poly(ethylene glycol) methyl ether methacrylate and poly(ethylene glycol) methyl ether acrylate were used to contrast the resulting gel properties with gels crosslinked with difunctional monomers.

After dissolving MHA, crosslinker, and the photoinitiator (10^{-3} *M*) in deionized water, mixtures were centrifuged for 1 minute at 850 rpm to eliminate any entrapped air bubbles. The solution was then pipetted into a rectangular silicon rubber mold (2 mm thick) adhered to a glass slide. After clamping the second glass slide on the top to keep out oxygen, samples were exposed to ultraviolet light of 312 nm (3.0 mW cm^{-2}) for 15 minutes on each surface inside a Spectrolinker XL-1000 (Spectronics Corp., Westbury, NY) photocrosslinker. Using a 3 mm biopsy punch, samples were then cut into disks and were equilibrated in deionized water for 24 hours before mechanical testing.

Swelling Ratio

The swollen samples were weighed and placed in an oven at 50°C to dry for 24 hours. The swelling ratio of the gels was calculated as the ratio of the swollen sample mass (m_s) to its corresponding dry mass (m_d):

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

The gel fraction (yield) was calculated as the ratio of dried polymer mass to polymer mass at gel formation:

$$\text{gel fraction} = \frac{m_d}{m_c \times \text{polymer mass fraction}} \quad (2)$$

in which m_c is the cylindrical sample mass before swelling.

Mechanical Testing

The compressive moduli (Young's (E) and shear (G)), fracture stress, and fracture strain of the hydrogels were determined under uniaxial compression of gel disks using a RSA-III dynamic mechanical analyzer (TA Instruments, New Castle, DE). Under a stereomicroscope (10×), the diameter of the swollen gel disks were measured using a caliper (0.01 mm resolution). The sample disk diameter and height ranged from 3.50 to 4.85 mm and from 2.65 to 3.40 mm depending upon the degree of swelling of each formulation. Samples were then loaded onto RSA-III. Compression plates were lubricated with mineral oil to reduce plate-gel adhesion. The tare load on the samples was 0.2 to 0.3 kPa. Quasi-equilibrium condition was maintained with a compression rate of 0.005 mms^{-1} in these experiments. The samples were pressed under compression until they began to split which was determined as the onset of 1 kPa drop in the recorded nominal stress value. Young's modulus of the samples was calculated by the slope of stress versus strain as shown below:[25,26]

$$\sigma = E\varepsilon \quad (3)$$

where $\varepsilon = \frac{L_0 - L}{L_0}$ and L and L_0 are the thickness of the deformed and undeformed specimen, respectively. In this study, the Young's modulus was calculated when $\varepsilon < 0.1$. Assuming the hydrogels as ideal elastomers, the shear modulus was calculated based on the neo-Hookean model as the slope of the stress versus strain function $\left(\lambda - \frac{1}{\lambda^2}\right)$ as shown below:[25,26]

$$\sigma = G\left(\lambda - \frac{1}{\lambda^2}\right) \quad (4)$$

where $\lambda = {}^{L}/_{L_0}$. In this study, the shear modulus was calculated when $\left(\lambda - \frac{1}{\lambda^2}\right) < 10$.

The crosslink density (ρ_x) of the samples was calculated based on the affine model of rubber elasticity from eq. (5) as follows:[7]

$$\rho_x = \frac{G}{RT\phi_2^{1/3}\phi_{2f}^{2/3}} \quad (5)$$

where R is the ideal gas constant, T is the absolute temperature, ϕ_2 is the polymer volume fraction in the swollen gel (inverse swelling), ϕ_{2f} is the polymer volume fraction at network formation, and ρ_x is the effective crosslink density in the polymer network (moles per volume polymer).

RESULTS AND DISCUSSION

Swelling Ratio and Mechanical Properties

Figure 1(A,B) shows the changes in swelling degree and shear modulus (eqs. (1) and (4)) of MHA 13 wt % gels with changes in crosslinker length (varying number of —CH_2CH_2O— repeat units). The molar concentration of the macromolecule disaccharide (0.52 mol %) and crosslinker (0.06 mol %) were kept constant throughout the experiment. This molar concentration was equivalent to 0.48 to 2 wt % of crosslinker (increasing with the crosslinker molecular weight) while the mass ratios of OEGDA : MHA was changed from 0.037 to 0.15.

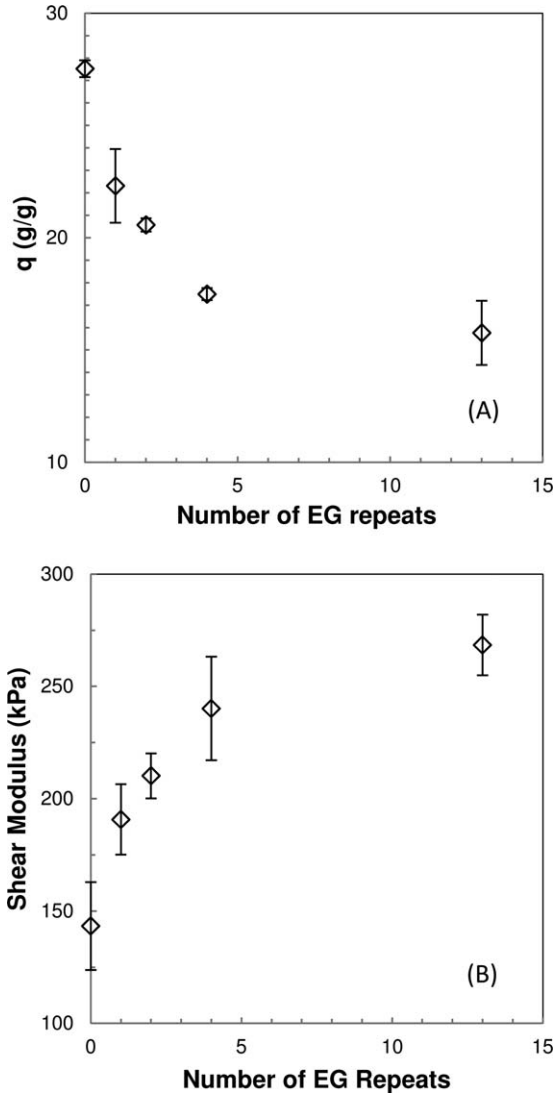

Figure 1. Swelling ratio (A) and shear modulus (B) of methacrylated hyaluronic acid 13 wt % (DM 47%)—oligo(ethylene glycol) diacrylate 0.06 mol % ($(EG)_nDA$) copolymer hydrogels. The swelling ratios of crosslinked MHA gels decreased as a function of the ethylene glycol segment length in crosslinker, while the shear modulus increased with the number of ethylene glycol repeat units in the crosslinker. Mean value ± standard deviation, $n = 5$.

As shown in Figure 1(A), the swelling ratio of the MHA homopolymer gel was the highest while addition of $(EG)_nDA$ reduced the swelling. After an initial reduction in swelling ratio when MHA was crosslinked with $(EG)_1DA$, the swelling ratio was lowered steadily and the swelling of $(EG)_{13}DA$ crosslinked gels was less than 40% of the parent homopolymer. The shear modulus of MHA homopolymer, Figure 1(B), was the lowest in the group. However, when MHA was copolymerized with $(EG)_1DA$, the shear modulus was increased by 35%. The shear modulus increased with increasing length of the ethylene glycol comonomer almost steadily, with MHA-$(EG)_{13}DA$ being the stiffest gel with a shear modulus of 270 ± 13 kPa.

Table I. Mechanical Properties of MHA 13 wt % (DM 47 mol %) Gels

Crosslinker	q (g/g)	Gel fraction	E (kPa)[a]	E/G[a]	Fracture stress (kPa)[a]	Fracture strain (%)[a]	ρ_x (mol m^{-3})
-	27.52 ± 0.22	0.83 ± 0.01	476 ± 63	3.33 ± 0.14	75.1 ± 9.7	16.28 ± 0.61	675.3 ± 1.8
$(EG)_1DA$	22.30 ± 0.53	0.88 ± 0.02	667 ± 56*,#	3.50 ± 0.01	85 ± 16	15.3 ± 1.6	818.0 ± 6.4*,#
$(EG)_2DA$	20.57 ± 0.60	0.90 ± 0.04	750 ± 31*,#	3.57 ± 0.10	103.2 ± 8.1	15.73 ± 0.59	873.2 ± 8.4*,#
$(EG)_4DA$	17.49 ± 0.27	1.00 ± 0.02	820 ± 75*	3.42 ± 0.04	138 ± 13	18.0 ± 2.2	932.3 ± 4.8*,#
$(EG)_{13}DA$	15.76 ± 0.37	1.00 ± 0.03	949 ± 30*,#	3.54 ± 0.14	145 ± 30	16.8 ± 2.5	957.2 ± 7.5*,#

[a] Values are mean ± standard deviation with $n = 5$.
Groups marked with * are statistically different from MHA 13% homopolymer ($P < 0.001$).
Represents statistically significant difference from the value of previous group in Table I ($P < 0.05$).

The calculated gel fraction [eq. (2)], Young's modulus (E) (eq. (3)), E/G ratio, fracture stress, fracture strain, and crosslink density (ρ_x) for Figure 1 gels are reported in Table I. The gel fraction was the lowest in MHA homopolymer demonstrating incomplete incorporation of the MHA macromer into the MHA gel. This effect was consistent with the higher swelling degree and lower shear and Young's modulus of MHA homopolymer. The close proximity of E/G value to 3 (Poisson's ratio of 0.5) validated[27] the ideal elastomer assumption for all copolymers, which was used in calculating the crosslink density of gels [eq. (5)]. As shown in Table I, the Young's modulus and fracture stress of the gels essentially followed the same trends as shear modulus. Both Young's modulus and fracture stress increased with increasing crosslinker spacer length, although the fracture strain of the gels remained between 15 to 18% in all cases despite changes in crosslinking density. In the calculated network properties, ρ_x increased steadily with increasing crosslinker length. The crosslink density of $(EG)_{13}$ crosslinked MHA was 1.4 times higher than MHA homopolymer.

Representative stress versus strain and stress versus strain function plots for the gels of Table I are presented in Figure 2(A,B) and the slopes of these plots were used to calculate the Young's and shear moduli of gels, respectively. MHA homopolymer had the lowest slopes and the lowest elastic and shear moduli. The addition of OEGDA comonomer increased the slope and longer oligomers resulted in higher moduli gels.

Based on Flory's classic theory, the swelling degree of a polyelectrolyte gel is determined from the balance between the osmotic pressures arising from polymer–solvent mixing, the presence of counter ions, and forces originating from the elastically effective junctions.[28] The relatively high swelling ratio of MHA homopolymer mainly resulted from association of counter ions with the ionized glucoronic acid residues of HA. However, as Figure 1(A) shows the high swelling ratio of HA can be significantly reduced by copolymerization with oligo(ethylene glycol) diacrylates. The presence of small, mobile OEGDA monomers during the reaction can overcome the steric hindrances of the polysaccharide structure and increase the crosslink density of the gels. Copolymerization can also reduce the charge density per unit mass and increases the average Flory–Huggins χ parameter of the copolymer gel, although the amount of OEGDA is small. The increase in moduli [Figure 1(B) and Table I] is also consistent with increased crosslink density [eq. (5)] and lowered

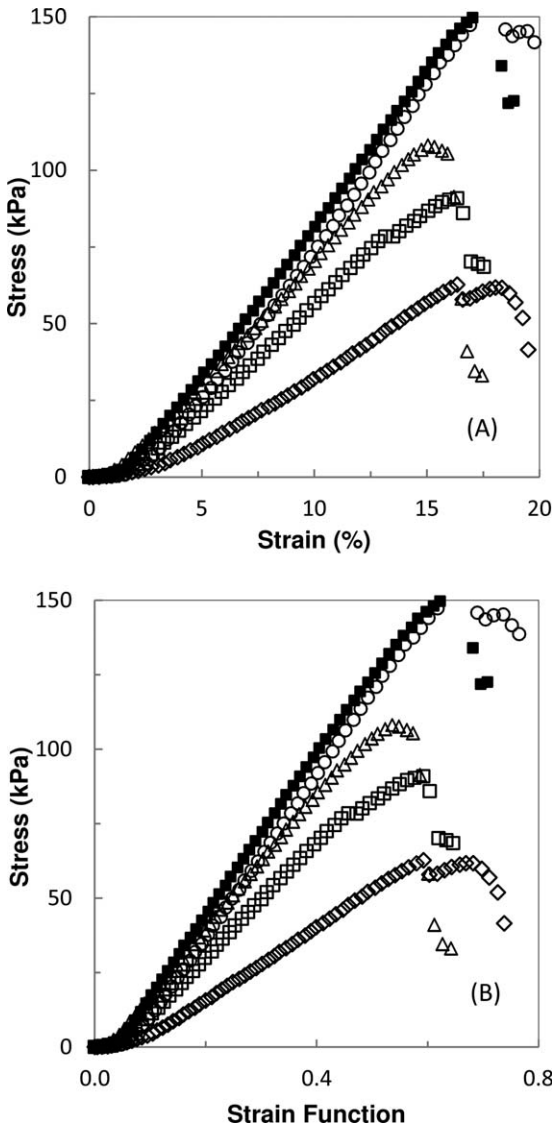

Figure 2. Representative stress versus strain (A) and stress versus strain function (B) plots for 13 wt % MHA (DM 47 mol %) gels. The slopes represent the Young and shear modulus of the gels and increased with the length of the comonomer. Symbols used to represent the gel types are as follows: homopolymerized MHA (◇) and MHA crosslinked with $(EG)_1DA$ (□), $(EG)_2DA$ (Δ), $(EG)_4DA$ (○), and $(EG)_{13}DA$ (■).

swelling of gels. The effect of crosslinker length on mechanical properties of the gels will be discussed in the following section.

Crosslinking by Formation of Kinetic Chains

Despite widespread application of photopolymerized glycosaminoglycan (GAG) hydrogels, the governing photopolymerization mechanism has not been fully explored. Understanding the photocrosslinking mechanism is essential since it would affect the morphology, the swelling and mechanical properties of the resulting gels. There are at least two potential photocrosslinking mechanisms to crosslink functionalized GAGs.[7] The first possible mechanism would be direct linking of macromers via the EG linker of the diacrylate crosslinker. The second possibility is covalent crosslinking of multiple methacrylate and acrylate groups into common kinetic chains. The former mechanism is similar to direct crosslinking methods (e.g., Michael addition using divinylsulfone) with more homogeneous composition while formation of kinetic chains in the latter mechanism could potentially result in heterogeneities associated with creation of relatively hydrophobic kinetic chains. To test the hypothesis that MHA crosslinking happens primarily via the formation of kinetic chains, a monoacrylate oligomer was used to crosslink the gels. Using a monoacrylate crosslinker would eliminate the possibility of directly connecting two macromer methacrylate groups. Poly(ethylene glycol) methyl ether acrylate ($(EG)_9MEA$) was used twice the molar concentration of the diacrylate crosslinker (0.12 *M* vs. 0.06 *M*) to ensure equimolar concentration of the acrylate groups in both systems. Moreover, to further test the effect of crosslinker functional groups, one mono- (monofunctional poly(ethylene glycol) methyl ether methacrylate $(EG)_9MEMA$) and one dimethacrylate crosslinker (tetra(ethylene glycol) dimethacrylate $(EG)_4DMA$) analogous to the acrylates, were copolymerized with MHA.

In Figure 3, the shear moduli of the resulting gels are compared. As shown in Figure 3, monoacrylate crosslinked MHA gel had an equally high shear modulus as $(EG)_4DA$ crosslinked gel. In contrast, both mono- and dimethacrylate crosslinked gels had lower moduli than homopolymer gels with dimethacrylate crosslinked gel as the weakest gel in the group. The increased shear modulus of MHA after crosslinking with a monoacrylate monomer confirmed the hypothesis that in this system crosslinking mainly happens via the formation of kinetic chains, as previously observed in MCS gels.[7] Therefore, the connection of two MHA molecules via the $(EG)_n$ spacer of the diacrylate crosslinker is not the main crosslinking mechanism here. Higher molecular weight diacrylate crosslinkers improve the crosslinking efficiency because both acrylate groups can participate in either common or different kinetic chains. However, in shorter diacrylates, the second group is less likely to be incorporated into elastically effective junctions as it is more probable to be involved in intramolecular reactions. Hence, the improvement in properties is believed simply due to the improved crosslinking effects rather than due to the known mechanical property enhancement of a bimodal distribution of long and short chains.[29,30] Since difunctional OEGDAs are more readily synthesized than the monoacrylates, difunctional monomers are used widely in crosslinking schemes. Moreover, lower molecular weight OEGDAs were used in this study to test the influence of

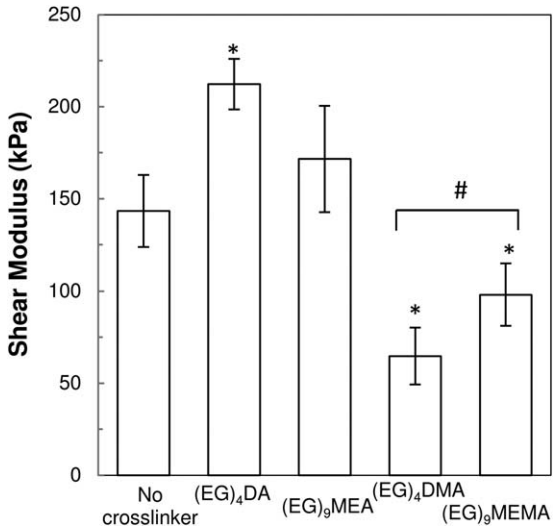

Figure 3. Shear moduli of 13 wt % MHA gels (DM 47 mol %) crosslinked with: poly(ethylene glycol) methyl ether acrylate ($(EG)_9MEA$), tetra(ethylene glycol) diacrylate ($(EG)_4DA$), poly(ethylene glycol) methyl ether methacrylate ($(EG)_9MEMA$), and tetra(ethylene glycol) dimethacrylate ($(EG)_4DMA$). Monofunctional monomers were used in twice the molar concentration of difunctional crosslinkers. Mono and diacrylate oligomers equally increased the shear modulus while mono and dimethacrylate analogues lowered the MHA modulus.* $P < 0.005$ versus MHA homopolymer; # $P < 0.05$ ($n = 5$).

the chain size. In practice, higher molecular weight PEGDA (2 kDa or 3.4 kDa) would be preferred for cell encapsulation applications as they are less cytotoxic.[31] Similarity of the findings of this study and our previous MCS study supports the hypothesized generality of this crosslinking mechanism in methacrylated polysaccharide gels. Hence, monomers other than ethylene glycol derivatives can be used by this scheme as long as they have desirable biocompatibility and suitable reactivity ratios, as will be discussed in the following section.

Reactivity Ratio Effect

As discussed previously, the greater shear moduli of MHA–OEGDA copolymers relative to MHA–OEGDMA copolymers (Figure 3) followed the trend observed in our previous study of methacrylated chondroitin sulfate gels.[7] This behavior can be explained by considering differences in reactivity ratios typically observed in methacrylate–acrylate copolymerizations. In these systems, the reactivity ratio for methacrylates is usually greater than one (~1.2–1.8), thus favoring homopropagation, while for acrylates, it is usually less than one (~0.2–0.8), thus favoring crosspropagation.[32,33] Since efficient crosslinking in this system is the result of incorporation of macromers and low molecular weight monomers into common kinetic chains, it stands to reason that a tendency toward crosspropagation will enhance the development of kinetic chains that incorporate both molecules, especially when the macromer is in excess. This concept has important implications for the selection of macromer and crosslinker functional groups, as it suggests that the macromer should be a methacrylate and the low molecular weight monomer should be an acrylate for the most efficient crosslinking. In this case, the acrylate monomer will favor reaction with the

methacrylated macromer over reaction with like monomers. A methacrylate monomers's tendency toward homopropagation would increase its consumption in homopolymer chains, leading to depletion before full incorporation into common kinetic chains. This will tend to be true even if the macromer is acrylated, especially considering the steric constraints on the macromer acrylate groups. It is logical to suggest that these concepts could be extended to other macromer/monomer combinations created by free radical copolymerizations.

Comparison Between Photopolymerized MHA and MCS Hydrogels

Our hypothesis that crosslinking efficiency of methacrylated polysaccharides is improved by copolymerization with low molecular weight comonomer has been confirmed by extending the concept from MCS to MHA. In both cases, introducing lower molecular weight OEGDAs increased the moduli and crosslink density of gels consistent with our hypothesis of crosslinking via common kinetic chains. The previously studied MCS gels with 24 and 34 mol % substitution degrees showed swelling ratios of 234 and 44 (*g*/*g*), respectively. The measured swelling ratio for MHA gels with changes in substitution degree in the literature shows a similarly sharp drop in swelling ratio from 93 to 38 (*g*/*g*) when the HA methacrylation increased from 14 to 23 mol %.[5] Therefore, the degree of methacrylation controls the swelling of gels as previously suggested.[7] Moreover, while MCS gel (13 wt %) with 34 mol % methacrylation yielded a crosslink density of 225 mol m^{-3}, 47 mol % methacrylated HA yielded 3 times higher density of 675 mol m^{-3}.[7] While the degree of substitution can be considered the main reason for these variations, structural differences between MHA and MCS can be another potential reason. Furthermore, both mono- and dimethacrylate crosslinkers suppressed crosslinking MHA gels as also observed in MCS gels (though this suppression in the case of MHA gels was not as significant as in MCS gels). In both MCS and MHA cases, increases in crosslink density did not change the fracture strain of the gels, suggesting that fracture strain may be a characteristic of the highly extended conformation of GAGs or the microheterogeneity of this type of gel, rather than the degree of crosslinking *per se*.

CONCLUSIONS

Hyaluronic acid hydrogels are one of the most important biopolymer gels used in biomedical applications. In biological applications of HA, fine-tuning mechanical properties of the gels to precise values without significant compositional changes is highly valuable. It is widely recognized in tissue-engineering applications that cell behavior is highly sensitive to the modulus of the gel scaffold. In this study, we showed how using even small amounts of oligo(ethylene glycol) acrylate (mass ratio of crosslinker : MHA from 0.037 to 0.15) can readily tune the swelling, moduli and crosslink density of MHA gels to desired values. Tuning the mechanical properties by this method will be much simpler than doing so by varying the polymer molecular weight, degree of substitution, or concentration. Additionally, since it can be difficult to avoid batch-to-batch variability of these substituted biopolymers, this study suggests a simple way to adjust for such variations by slight changes in comonomer ratio. Additionally, this technique could be used to create well-crosslinked gels from more lightly modified hyaluronic acid macromers, which would help maintain the desirable biological activity of HA. Higher molecular weight PEG-acrylates that have better cell compatibility than the OEGDAs could be used with the same strategy to design biomaterials with improved mechanical properties for cell encapsulation purposes.

ACKNOWLEDGMENTS

This study was supported by Grant DMR 0805264 from the National Science Foundation. Any opinions, findings, and conclusions or recommendations expressed in this article are those of the authors and do not necessarily reflect the views of the National Science Foundation.

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