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Decoupling the dependence of rheological/mechanical properties of hydrogels from solids concentration

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Abstract

Hydrogels are being increasingly used in medicine due to their potential to be delivered into the body in a minimally invasive manner (e.g. injection in solution form) for subsequent gelation at the site of introduction. Both the flow properties of the solution and mechanical properties of the gels are critical to their applications. However, both properties depend on the concentration of polymer, and simply increasing the concentration to improve the gel properties often leads to unacceptably high fluid viscosities. Thus, we hypothesized that hydrogels with a bimodal molecular weight distribution (MWD) (i.e. a mixture of high MW polymer and polymer tailored to have a lower MW but still able to participate in gel formation) would allow one to readily decouple the dependence of the two properties from the overall concentration. This hypothesis was investigated using alginate hydrogels, and we found that increasing the concentration of alginate (C_{alginate}) with high MW alginate from 2 to 5% raised the viscosity (η) of solution from 0.7 to 20 Pa s, while enhancing the shear modulus (G) from 25 to 50 kPa. In contrast, increasing C_{alginate} of binary solutions at a weight fraction of high MW alginate of 0.50 raised η from 0.2 to only 3.6 Pa s, while enhancing G from 15 to 52 kPa. Strikingly, the low η of binary solutions can be attributed to a significant decrease in physical interactions between the chains, while strong gel strength could be attributed to an increased fraction of intermolecular cross-links and stiffened molecules as compared to gels comprised of high MW alginates. This approach of adjusting the MWD of gel forming solutions to control the fluid and solid properties in an independent manner may be broadly utilized in designing other hydrogels and materials for a variety of applications. © 2002 Published by Elsevier Science Ltd.

Keywords: Binary hydrogel; Alginate; Chain stiffness

1. Introduction

Many advantageous features of hydrogels (i.e. good biocompatibility, low interfacial tension, and similarity to the hydrated macromolecular components of the body) have led to their use as biomaterials to carry drugs and bioactive macromolecules into the body [1–3]. Recently, hydrogels have been applied as three-dimensional transplantation vehicles for cells in soft tissue engineering, where they are designed to imitate the functions and properties of extracellular matrices, and aid in the regeneration of damaged tissues or organs [4]. In particular, the readily controlled gelation kinetics of hydrogels makes them attractive for minimally invasive surgical procedures due to their injectability [5].

To successfully utilize hydrogels as injectable delivery vehicles, it is important to control the fluid properties of the pre-gelled solution and the mechanical properties of the post-gel. The fluid properties (i.e. viscosity) are critical to the delivery of the material (e.g. flow through a syringe needle or endoscope). In addition, inappropriate fluid properties (e.g. viscosity too high to allow complete mixing) may inhibit the ultimate performance of the gel. The mechanical properties of the gelled material in the body (i.e. rigidity and brittleness) are critical to maintain the gel structure in the face of the compression from neighboring tissues, and provide a space for new tissue formation [6]. In addition, the mechanical stiffness can potentially be utilized to regulate the gene expression of the cells distributed in hydrogels [7]. Therefore, it may be highly advantageous to

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attain a broad range of mechanical properties from hydrogels, while maintaining suitable flow properties of the pre-gelled solution.

Hydrogels formed from sodium alginate, a natural polysaccharide derived from brown algae, have many attractive features for biomedical applications. Due to their biocompatibility and low toxicity, these hydrogels have already been used as wound dressings, drug and cell encapsulation materials, and injectable cell transplantation vehicles [8,9]. Alginate is composed of blocks of β-D-mannuronic acid residues (MM-blocks), blocks of α-L-guluronic acid residues (GG-blocks), and blocks with alternating M and G residues (MG-blocks). Sodium alginate is freely soluble in water, and easily forms hydrogels by selective binding of carboxylic groups on G-blocks with divalent calcium ions [10].

The mechanical properties of elastic materials formed by cross-linking, including alginate, are generally determined by the cross-linking density and the properties of main polymer chains/cross-linking molecules (i.e. molecular weight, chain stiffness) [11]. In this same context, the mechanical stiffness of ionically cross-linked alginate hydrogels has been controlled by adjusting the M/G ratio [12], the molecular weight of alginate [13], and/or concentrations of the binding cations [14]. Perhaps, the most straightforward method to vary the mechanical stiffness of alginate hydrogels is to increase the concentration of alginate in the solution used to form a gel, irrespective of the type of cross-linking. However, increasing the concentration of the high MW alginate typically used to form hydrogels may greatly increase the viscosity of the pre-gelled solution prior to gelation. Processing and material reliability issues may significantly limit this approach. Alternatively, preparing hydrogels with a high concentration of low MW alginate may limit the increase of the viscosity, while enhancing the stiffness of the hydrogel due to the increase of the solids concentration. However, this approach may not be ideal, due to the potential brittleness of the resultant gels.

We hypothesized that a general approach to decouple the dependence of the rheological properties of a pre-gelled solution and the mechanical stiffness of the post-hydrogel from the solids concentration would be to adjust the molecular weight distribution (MWD) of the polymer used to form a gel. A complication to this simple approach with alginate is that simply combining commercially available high MW alginate with low MW alginate to obtain a bimodal MWD may not be a desirable approach, as the length of GG-blocks in the alginate chains cannot be lower than a critical length required to allow ionic cross-linking [15]. This study thus specifically addressed our hypothesis by generating low MW alginate chains from high MW alginate, while maintaining a consistent GG-block component to the gel.

2. Experimental work

2.1. Materials

Sodium alginate rich in GG-blocks ($M_n = 2.6 \times 10^5$ g/mol, $M_w = 2.7 \times 10^5$ g/mol) supplied from FMC Technologies was used as the high molecular weight (MW) component. Calcium sulfate (Aldrich), sodium chloride (J.T. Baker), and sodium hexametaphosphate (Johnson Matthey) were used as received. Alginate was broken into low MW chains using gamma (γ)-irradiation. Alginate powder was irradiated with a cobalt-60 source for 4 h at a γ-dose of 5.0 Mrad. Dulbecco's modified Eagle's medium (DMEM) was purchased from Life Technologies. All materials were sterilized prior to use by filtration through 0.22 μm filters or autoclaving.

2.2. Characterization of alginate molecules

Molecular weights and molecular weight distributions of alginate samples were determined with a size-exclusion chromatographic system equipped with a triple detector system (Viscotek) including a laser refractometer (LR 40), a differential viscometer (T60), and a right angle laser light scattering detector (RALLS). 0.1 M $NaNO_3$ buffer solution (pH 6.3) was used as a mobile phase and the samples were dissolved in the mobile phase, and injected through a Rheodyne valve equipped with a 100 μl injection loop. A set of two TSK-gel columns ($G4000PW_{XL}$ and $G3000PW_{XL}$) was used to separate the alginate molecules with a different molecular weight.

To quantify the stiffness of semi-flexible alginate molecules, Kuhn's segment length (b_k) and molecular weights per contour length (M_L) were calculated using Eqs. (1)–(3)), following the procedure proposed by Bohdanecký [16,17]. Since alginate chains are comprised of chemically identical saccharide rings, it was regarded as a homopolymer in this calculation. For this calculation, experimentally measured $(M_w^2/[\eta])^{1/3}$ is linearly related to $M_w^{0.5}$, where $[\eta]$ is the intrinsic viscosity.

$$(M_w^2/[\eta])^{1/3} = A_\eta + B_\eta M_w^{0.5} \tag{1}$$

$$A_\eta = \Phi_\infty^{-1/3} A_0 M_L \tag{2}$$

$$B_\eta = \Phi_\infty^{-1/3} B_0 (M_L/b_k)^{0.5} \tag{3}$$

Φ_∞ is the Flory–Fox constant ($= 2.86 \times 10^{23}$ mol^{-1}). A_0 in Eq. (2), which depends on the hydrodynamic diameter (d), is calculated to be 1.0 from the following Eq. (4), and B_0 in Eq. (3) is assumed to be 1.05.

$$\log(d^2/b_k^2 A_0) = \log[(4\Phi_\infty/1.215\pi N_A)(v/A_\eta)B_\eta^4]$$

$$= 0.173 + 2.158 \log(d/b_k) \qquad \text{at } 0.1 \le d/b_k \tag{4}$$

where v is the specific volume of polymer (1.1 cm^3/g for alginate) [18].

M/G ratio of alginate was determined with a circular dichroism (CD) spectrometer (AVIV 202). The sample concentration was 0.4 mg/ml and absorbance of the solution was scanned at wavelengths from 250 to 190 nm at 25 °C. From the CD spectra, molar ellipticity values were acquired in units of deg cm^2/dmol. The M/G ratio was calculated by dividing the height of the peak (i.e. MM-blocks at 200 nm) by that of the trough (i.e. GG-blocks at 220 nm), since both the values were negative [19].

2.3. Preparation of ionically cross-linked alginate hydrogels

Alginate was dissolved in deionized water to yield solutions ranging from 2 to 5%(w/w) alginate. Note that gels are described by the weight percentage of solids throughout this paper. The weight fraction of high MW alginate [$W(H)$] as a fraction of the total solids was varied from 0 to 1. Alginate solutions were mixed with $CaSO_4$ slurries to make homogeneous mixtures, and the mixtures were cast between glass plates with a spacer of 2 mm thickness. After 2 h, gels were cut into disks with diameter of 12.7 mm and incubated in DMEM at 37 °C until mechanical testing. The molar ratio between carboxylic groups of alginate chains and calcium was kept constant at 1:0.6 in all gels, and 0.14 M NaCl and $Na_6(PO_3)_6$ were incorporated in the pre-gelled solutions to avoid an abrupt gelation process. The molar ratio between $Na_6(PO_3)_6$ and alginate was kept constant at 0.08:1 in all components.

2.4. Characterizations of pre-gelled solution and post-hydrogel

A controlled stress rheometer (CS-50, Bohlin Instrument) was used to measure the viscosity of alginate solutions at 25 °C. Prior to the measurement, all samples were pre-sheared at a high shear rate followed by rest for 5 min. The samples and loading apparatus were completely covered with a solvent trap to prevent the evaporation of water during the measurement. From the shear-thinning curve, the low shear viscosities (η), which was nearly independent of shear stress, were compared, as they reflect the molecular interactions in the pre-gelled solution at a low disturbance.

The reduced viscosity (η_{red}) was calculated by normalizing η with the solids concentration ($C_{alginate}$) and the viscosity of the solvent (η_0) (= 1 mPa s for water), as follows:

$$\eta_{red} = \frac{\eta - \eta_0}{\eta_0 \phi_{alginate}} \quad (5)$$

The post-hydrogels were compressed at a constant deformation rate of 1 mm/s with a mechanical tester (MTS Bionix 100, MTS systems) at 25 °C, and the resulting stresses were measured. From the stress vs. strain curves, the compressive elastic moduli of the hydrogels were calculated. Since the curves tended to be non-linear, the strain range used for the calculation of the moduli was limited to the first 10% of strain. Assuming that the alginate hydrogels fit to an affine network model, the shear modulus (G) was obtained from the slope σ vs. $-(\lambda - \lambda^{-2})$ plot, where σ is the stress, and λ is the ratio of the deformed length to the undeformed length of the hydrogel [11,20,21].

Gels incubated in DMEM at 37 °C were weighed to determine the degree of swelling of the gels. The water absorbed into the gel was subsequently removed by freeze-drying the gel, and the weight of dried gel was measured. The degree of swelling (Q) was defined as the reciprocal of the volume fraction of a polymer in a hydrogel (ν_2)

$$Q = \nu_2^{-1} = [(1/\rho_p)[Q_m/\rho_s + 1/\rho_p)]^{-1}]^{-1} \quad (6)$$

where ρ_p is the polymer density (0.8755 g cm^{-3}), ρ_s is the density of water, and Q_m is the swelling ratio, defined as the mass ratio of absorbed water to the dried gel.

From G and Q, the effective number of cross-links (N_0) and molecular weights between cross-links (M_c) were determined, based on the rubber elasticity theory [22]

$$N_0 = GQ^{1/3}/RT = (\rho/M_c)(1 - 2M_c/M_n) \quad (7)$$

where R is the gas constant (8.314 J mol^{-1} K^{-1}), T is the temperature at which the modulus was measured, and M_n is the number average molecular weight. The M_n of alginates in the binary system was calculated simply by summing up the products of weight fraction of low and high MW alginates and corresponding M_n [23].

3. Results

3.1. Characterization of irradiated alginate

The low MW alginate for use in the binary system was desired to have a MW lower than the renal clearance of the kidney ($\sim 80 \times 10^3$ g/mol), while maintaining a similar length of GG-blocks as the high MW alginate. For this purpose, commercially available alginate ($M_w = 2.7 \times 10^5$ g/mol) was irradiated at a gamma dose of 5 Mrad to obtain a M_w of 53,100 g/mol (Table 1). Calculations of the Kuhn's segment length (b_k) and molecular weights per contour length (M_L) with Eqs. (1)–(4)) revealed that irradiating alginate in this manner doubled

Table 1
Structural parameters of non-irradiated and irradiated alginates

γ-dose (Mrad)	M_w[a]	b_k[b] (nm)	M_L[c] (nm^{-1})	M/G[d]
0	269,100	7.8	(195	0.62
5	53,100	16.1	412	0.58

[a] Molecular weight distribution index.
[b] Kuhn's segment length.
[c] Molecular weights per contour length.
[d] Ratio of MM-blocks to GG-blocks.

both b_k and M_L, while reducing M_w by almost one order of magnitude (Table 1). The b_k of irradiated alginate chains is comparable to the reported b_k of other polysaccharide chains, (e.g. nitrocellulose; $b_k = 17$ nm) [24]. This result indicates that the low MW alginate has a less coiled conformation due to the higher chain stiffness, represented by the higher b_k and M_L. The significant change in the chain stiffness infers a selective breakdown of the polymer blocks in the alginate of lesser stiffness. The stiffness of the blocks comprising alginate decreases in the order of polyguluronic acid (GG-blocks), polymannuronic acid (MM-blocks), and blocks with alternating M and G blocks (MG-blocks) [25]. To further characterize the conformational changes, the CD spectra of non-irradiated and irradiated alginates were compared. This analysis indicates negligible changes in the M/G ratio occurred with irradiation, despite the reduction of MW (Table 1). This finding of a constant M/G ratio with the increases of b_k and M_L indicates that chain scission of polymer backbones mainly occurred within MG-blocks, while the structures of the other two stiffer blocks were maintained. Thus, irradiating alginate at a 5 Mrad dose does not appear to affect the length of single GG-blocks of the alginates, although it reduces the total number of GG-blocks on a single alginate chain.

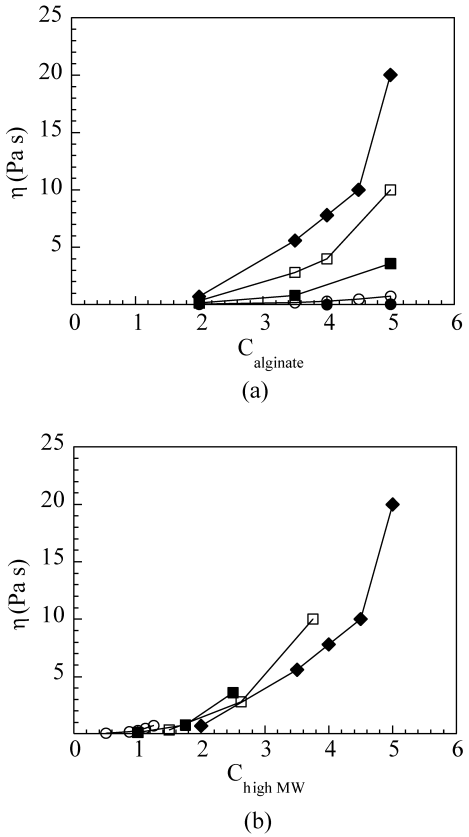

Fig. 1. Effect of total alginate concentration ($C_{alginate}$) on the low-shear viscosity (η) of solutions at a weight fraction of high MW alginate [$W(H)$] in total solids of 0 (●), 0.25 (○), 0.50 (■), 0.75 (□), and 1.00 (◆). (a) η vs. $C_{alginate}$. (b) η vs. concentration of high MW alginate fraction ($C_{high\ MW}$) only in the binary solution.

3.2. Control of rheological properties of pre-gelled solution

The viscosity of a polymer solution is dependent on the concentration of a polymer, its MW, and consequent interactions between polymer molecules (i.e. entanglements). To determine how the stiffer low MW alginate constrains the increase of the viscosity of pre-gelled solutions with an increasing concentration, the total solids concentration ($C_{alginate}$) was varied from 2 to 5%, and the weight fraction of high MW alginate [$W(H)$] in the total solids was varied from 0 to 1. Increasing $C_{alginate}$ generally resulted in an increase of the low-shear viscosity (η) of the pre-gelled solution (Fig. 1(a)). In addition, raising $W(H)$ led to a stronger dependence of the viscosity on $C_{alginate}$. Re-plotting Fig. 1(a) as a function of high MW alginate concentration ($C_{high\ MW}$) alone largely collapsed these distinct curves (Fig. 1(b)). This result indicates that $C_{high\ MW}$, not $C_{alginate}$, is the main factor determining the viscosity of these alginate solutions, and suggests very high loadings of $C_{alginate}$ with low MW chain in the solution still allows low and well-usable viscosities.

To gain insight into changes in the interactions between alginate molecules due to the changes of $C_{alginate}$ and $W(H)$, the reduced viscosity (η_{red}) was calculated, following Eq. (5). This η_{red} represents the magnitude of physical

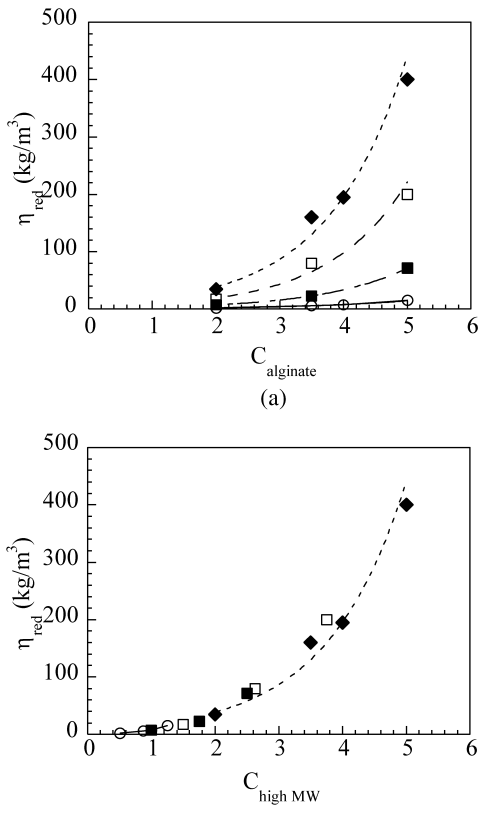

Fig. 2. Effect of $C_{alginate}$ on the reduced viscosity (η_{red}) of pre-gelled solutions at a $W(H)$ of 0.25 (○), 0.50 (■), 0.75 (□), and 1.00 (◆). (a) η_{red} vs. $C_{alginate}$. (b) η_{red} vs. $C_{high\ MW}$.

interactions between molecules by normalizing the contribution of the number of polymer molecules to the viscosity (Fig. 2(a)). When these η_{red} curves are re-plotted as a function of $C_{high\ MW}$, the curves collapse into one universal curve (Fig 2(b)). This finding confirms that physical interactions between high MW alginates mainly determine η_{red} of binary solution, and their interactions become stronger with increasing $C_{high\ MW}$.

3.3. Control of mechanical properties of post-gel

The stiffness and cross-linked structures of the hydrogels were next analyzed, following ionic cross-linking. Unary gels composed of solely high MW or low MW alginate were first studied. In general, increasing $C_{alginate}$ increased the shear moduli (G) of unary hydrogels in a linear manner, irrespective of the MW of the alginate hydrogels (Fig. 3(a)). Unary high MW hydrogels, however, displayed a decrease of G, as $C_{alginate}$ exceeded 4.5%. This is likely caused by an undesirable cross-linked structure or undesirable processing condition leading to the non-uniform mixing of calcium slurry in the pre-gelled solution. Hydrogels with a high stiffness could also be achieved from the low MW alginate solution with low viscosity by increasing $C_{alginate}$ (e.g. 5%). While unary low MW hydrogels could exhibit high stiffness, they tended to be highly brittle (Fig. 3(b)). Although increasing $C_{alginate}$ to 5% made small increase of the ultimate strain at failure, it was still much lower than that of unary high MW hydrogels. This brittleness suggests these materials would fail in many applications due to high strains imposed on the material in the body.

The mechanical properties of binary hydrogels formed by combining low and high MW alginates were next examined to determine if they could demonstrate a mixture of high stiffness, while maintaining good malleability. These gels also displayed an increase of modulus upon increasing $C_{alginate}$ (Fig. 4(a)). Increasing $W(H)$ or $C_{alginate}$ made the binary hydrogels not fail until the compression load reached the maximum loading capacity of the MTS system used (i.e. 10 N). However, incorporating small amounts of high MW alginate greatly enhanced the strain at failure of gels mainly composed of low MW alginate (Fig. 4(b)). These results indicate that binary hydrogels allow one to readily attain the desired stiffness by increasing

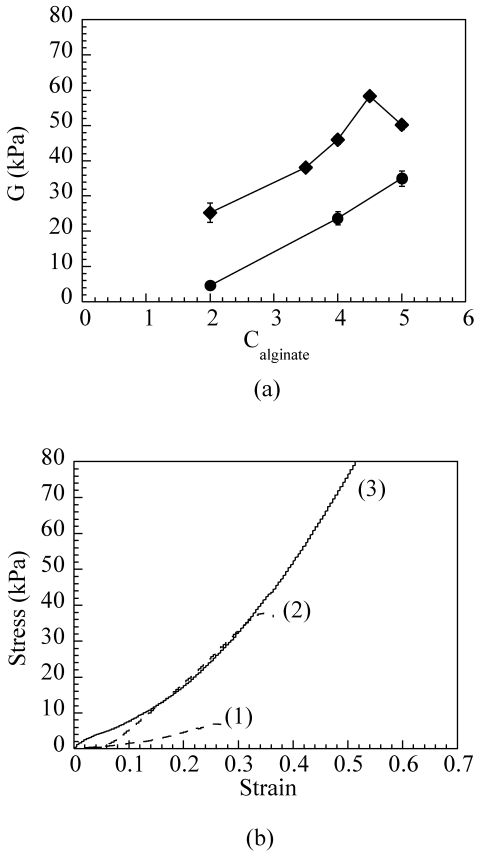

Fig. 3. Mechanical properties of unary hydrogels as a function of $C_{alginate}$. (a) Elastic shear modulus (G) of gels formed from low MW alginate (●) and high MW alginate (◆). (b) Typical stress vs. strain curves for 2% low MW hydrogel (curve 1), 5% low MW hydrogel (curve 2), and 2% high MW hydrogel (curve 3), demonstrating the low strain at failure of unary gels comprised of low MW alginate chains.

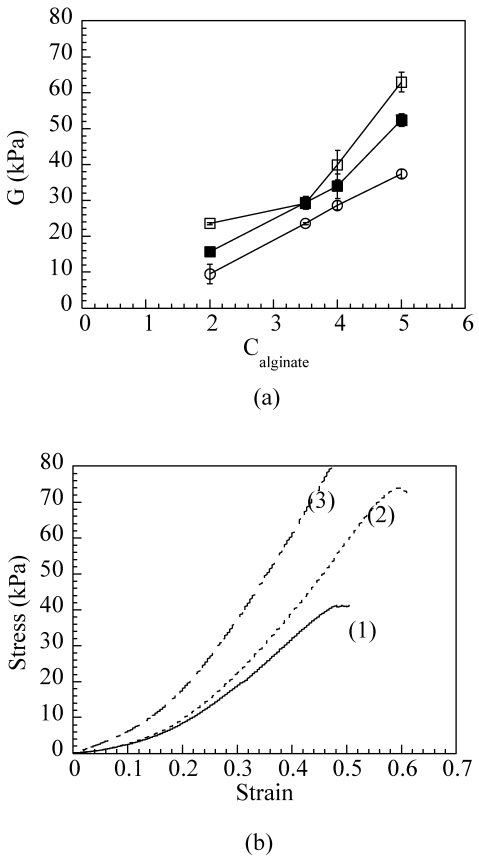

Fig. 4. Effect of $C_{alginate}$ on the mechanical properties of binary hydrogels. (a) Shear modulus (G) of hydrogels as a function of $C_{alginate}$ at a $W(H)$ of 0.25 (○),0.50 (■), and 0.75 (□). (b) Typical stress vs. strain curves of 2% hydrogel at a $W(H)$ of 0.25 (curve 1) and 0.50 (curve 2), and 3.5% hydrogel at $W(H)$ of 0.25 (curve 3), demonstrating the high strain at failure of binary gels.

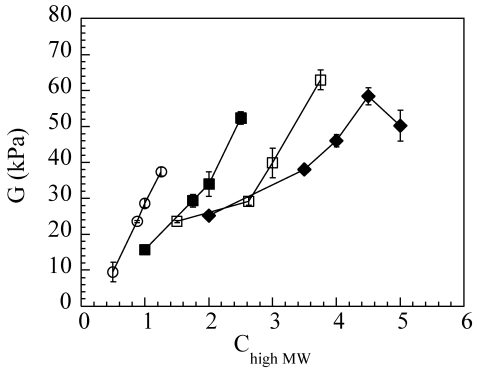

Fig. 5. Effect of $C_{\text{high MW}}$ on the shear modulus (G) of hydrogels at a $W(H)$ of 0.25 (○), 0.50 (■), 0.75 (□), and 1.00 (◆).

C_{alginate}, while preventing the hydrogels from becoming extremely brittle. Re-plotting the modulus vs. C_{alginate} data (Figs. 3(a) and 4(a)) as a function of $C_{\text{high MW}}$ disclosed that much lower $C_{\text{high MW}}$ was required to attain a given modulus, as $W(H)$ was lowered (Fig. 5). Low MW alginate clearly contributes to improving the stiffness of the binary hydrogels, and thus the role of low MW alginate on the stiffness of the gel is contrasted to its insignificant contribution to the viscosity of the pre-gelled solution. This result illustrates that addition of low MW alginate could successfully decouple the dependences of the properties of the pre-gelled solution and post-gel from C_{alginate}.

To further investigate the cross-linked structures of hydrogels, the degree of swelling (Q) was related to the shear modulus (G) (Fig. 6) [26]. Q linearly decreased with increasing G on a log scale, indicating that ionically cross-linked hydrogels follow the Gaussian elastic networks model. However, the dependence of G on Q became stronger with decreasing $W(H)$. In addition, high MW hydrogels displayed an inflection of G following the initial increase, corresponding to the continuous decrease of Q. These findings indicate the development of intramolecular cross-links in the unary high MW hydrogels with increasing C_{alginate}, as G reflects only the intermolecular cross-links

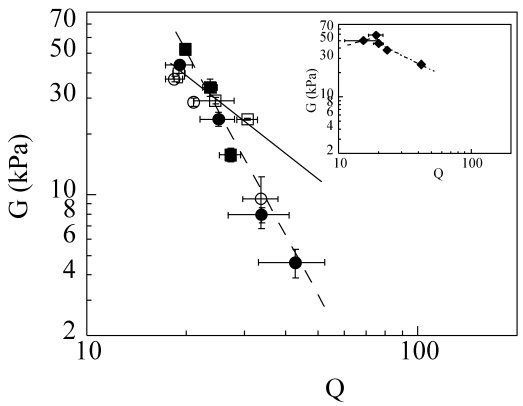

Fig. 6. Relation between shear modulus (G) and the swelling ratio of gels (Q) as a function of $W(H)$ values of 0 (●), 0.25 (○), 0.50 (■), 0.75 (□), and 1.00 (◆). The inserted box displays the relation between G and Q at a $W(H)$ of 1.00 only.

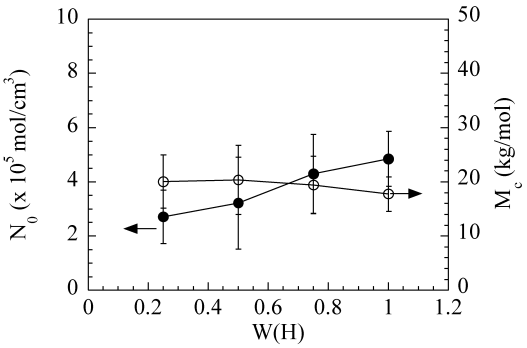

Fig. 7. Effect of $W(H)$ on the effective number of cross-links (N_0) (●) and molecular weights between cross-links (M_c) (○) in binary hydrogels.

providing the elastic response, while Q reflects both inter- and intramolecular cross-links [27]. Importantly, these data infer that decreasing $W(H)$ constrains the formation of intramolecular cross-links. This suggests that one can more efficiently increase G with raises in C_{alginate} in the binary system than in the unary high MW system.

To further relate the macroscopic responses of the gels to their cross-linked structure the number of effective cross-links (N_0) and molecular weights between cross-links (M_c) were calculated, following Eq. (7). Since log G is linearly related to log Q, as shown in Fig. 6, the cross-link density of the hydrogels could be calculated based on Gaussian elasticity network theory. Interestingly, N_0 and M_c are not strongly dependent on $W(H)$ (Fig. 7), despite a reduction in the average molecular weight of the chains comprising the gel with decreasing $W(H)$. This result is in contrast to models of physically cross-linked binary hydrogels, which predict that inclusion of low MW polymer increases the fraction of sol, due to a lack of involvement of the low MW chains in the cross-linked network [28]. In contrast, the calculations with the present system demonstrate that low MW alginate molecules did not induce these structural imperfections, likely because these polymer chains retained the structure of GG-blocks required for participation in ionic cross-linking.

4. Discussion

This study has demonstrated that polymer solutions with a bimodal molecular weight distribution successfully decouple the dependences of the viscosity of the pre-gelled solution and the mechanical stiffness of the post-gel from C_{alginate}. Low MW alginate, which was tailored to maintain a capability to form gels, made a negligible contribution to the viscosity of pre-gelled solution, while it significantly contributes to the stiffness of hydrogels with increasing C_{alginate}. In addition, a small fraction of high MW alginate in binary hydrogels maintained a high strain at failure. These phenomena can be attributed to the different roles of low MW alginate in the pre-gelled solution and post-gel (i.e. it does not enhance the physical interactions between

molecules in the solution, while it is highly effective in cross-linking the gel). A particularly striking finding is that use of a binary MW distribution constrained the formation of intramolecular cross-links upon increasing $C_{alginate}$ in this system.

The lack of a significant dependence of the solution viscosity on the concentration of low MW alginate can be interpreted in the context of the relaxation time of the polymer chains in the solution. Relaxation time is defined as the time for one molecule to become dissociated from physical interactions with neighboring molecules, and the effect of interactions between polymer molecules on a solution viscosity can be qualitatively interpreted in terms of a relaxation time of the polymer chains. The relaxation time generally depends on the size, stiffness, and total numbers of chains in a solution. High MW alginate, which is bulkier and more flexible than low MW alginate, physically interacts with other neighboring high MW alginates (i.e. physical entanglements). The enhanced physical interactions resulted in an increase of the relaxation time, which was found to increase in proportion to the number of high MW polymeric molecules. In contrast, the stiffer and shorter low MW chains avoid strong physical interactions, even with the neighboring high MW alginates in a binary solution, decreasing the dependence of the relaxation time on $C_{alginate}$. Interestingly, however, the universal η_{red} vs. $C_{alginate}$ curve for these solutions infers that low MW alginate molecules do not interfere with the physical interactions between high MW molecules. Note that the concentration ranges adopted in this study exceed an overlap concentration necessary to start the physical interactions between molecules.

The mechanical characteristics of the cross-linked gel can also be qualitatively related to the interactions between alginate molecules in the solution. At high $C_{alginate}$, strong physical interactions between high MW alginates greatly decrease the mobility of these alginate molecules. As a consequence, these flexible high MW alginate chains are more liable to form intramolecular cross-links along a single molecule. In contrast, the stiffer and shorter low MW alginate has a more stretched conformation than the high MW alginate, and this improves the formation of intermolecular cross-links, in contrast to intramolecular cross-links. However, the rupture of unary low MW hydrogels at low strain indicates a poor capacity to transfer loading energy to neighboring cross-linked domains, as compared to unary high MW hydrogels. In unary low MW gels, the stress is likely localized in the less densely cross-linked domains, and increasing $C_{alginate}$ with low MW alginate does not appear to reduce the stress localization.

Combining high and low MW alginates to form binary hydrogels realizes the advantageous features of both high MW and low MW hydrogels. First, the increase in the modulus of binary hydrogels with increasing $C_{alginate}$, at all values of $W(H)$ above 0, indicates that the low MW alginate effectively compensates for the reduced amount of high MW alginate and allows one to attain a hydrogel with high stiffness. Indeed, the inclusion of low MW chains actually reduces the fraction of intramolecular cross-links, compared with unary high MW hydrogel at a given $C_{alginate}$ (Fig. 6). Second, the pronounced increase in the strain at failure upon incorporation of small fraction of high MW alginate (Fig. 4(b)) infers a favored formation of intermolecular cross-links between high and low MW alginates. This improved the capability of gels to transfer deformation energy throughout the entire hydrogel.

In conclusion, these results indicate that adjusting MWD with properly tailored alginate molecules can independently control the microstructures of pre-gelled solutions and post gel material, and thus improve the fluid and mechanical properties altogether upon increasing $C_{alginate}$. This approach may be widely useful for a variety of hydrogels and other materials.

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