Controlling Rigidity and Degradation of Alginate Hydrogels via Molecular Weight Distribution

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The mechanical rigidity and degradation rate of hydrogels utilized as cell transplantation vehicles have been regarded as critical factors in new tissue formation. However, conventional approaches to accelerate the degradation rate of gels deteriorate their function as a mechanical support in parallel. We hypothesized that adjusting the molecular weight distribution of polymers that are hydrolytically labile but capable of forming gels would allow one to alter the degradation rate of the gels over a broad range, while limiting the range of their elastic moduli (E). We investigated this hypothesis with binary alginate hydrogels formed from both ionically and covalently cross-linked partially oxidized (1% uronic acid residues), low [molecular weight (MW) ∼ 60 000 g/mol] and high MW alginates (MW ∼ 120 000 g/mol) in order to examine the utility of this approach with various cross-linking strategies. Increasing the fraction of low MW alginates to 0.50 maintained a value of E similar to that for the high MW alginate gels but led to faster degradation, irrespective of the cross-linking mode. This result was attributed to a faster separation between cross-linked domains upon chain breakages for the low MW alginates, coupled with their faster chain scission than the high MW alginates. The more rapidly degrading oxidized binary hydrogels facilitated the formation of new bone tissues from transplanted bone marrow stromal cells, as compared with the nonoxidized high MW hydrogels. The results of these studies will be useful for controlling the physical properties of a broad array of hydrogel-forming polymers.

Introduction

Biomaterials are an essential component of various medical therapeutics such as drug delivery systems and wound treatments.1 The physical properties of biomaterials, including mechanical properties and degradation behavior, should be readily controlled over a broad range to match the requirements for a particular application. For example, in tissue engineering one typically desires to reproduce the structure and function of lost or damaged tissues or organs.2 An independent control over various physical properties of the materials may be required for a single material to act as both a good mechanical support and a template to guide new tissue formation. However, most biomaterials formed from cross-linking of polymeric precursors, such as hydrogel-based materials, exhibit a parallel dependence of degradation rate and elastic moduli on the degree of cross-linking. Methods that allow the degradation rate of the gels to be modified without a significant change in their elastic moduli could be broadly useful.

Calcium cross-linked sodium alginate hydrogels are utilized in various biomedical applications3 and slowly degrade in physiological conditions due to the gradual exchange of calcium,4 which cross-links guluronic acid (G) blocks, for monovalent ions. No hydrolytic or enzymatic chain breakages occur within alginate chains under physiological conditions.5 Further, alginate hydrogels formed from covalent cross-linking6 show much higher stability over time as compared with calcium cross-linked gels. To adjust the degradation rate of alginate gels, we have previously developed a technique to induce hydrolytically labile acetal-like groups within the alginates via an oxidation reaction.7,8 The degradation rate of the gels could be accelerated in proportion to the number of oxidized uronic acid residues. However, oxidation also made the gels more malleable, leading to an inverse relation between degradation rate and gel stiffness.8

We hypothesized in this study that adjusting the molecular weight distribution (MWD) of oxidized alginates may allow us to alter the degradation rate of the gels without varying the number of oxidized uronic acids, as long as the modified alginates maintain their chain inflexibility and gel-forming ability. This could allow one to control the elastic moduli and degradation rates of these gels in an independent manner. To examine this hypothesis, we utilized two partially oxidized alginates having a 2-fold difference in molecular weight (MW) as a model system. The low MW alginates were generated from the high MW alginates in a manner that does not decrease the length of G blocks that are exclusively involved in ionic cross-linking.3 The oxidation

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of both alginate polymers were then conducted under conditions that do not decrease the stiffness of the polymer molecules. Gels cross-linked ionically or covalently using carbodiimide chemistry as previously described were examined in this study to determine if degradation of both gel types could be controlled. The utility of these degradable gels in the formation of new tissues was studied using the transplantation of bone marrow stromal cells.

Experimental Section

Modification and Characterization of Alginate Molecules. Molecular weight (MW) of alginites rich in guluronic acid (G) blocks (MVG, FMC Technologies) was decreased by irradiating solid granules of sodium alginate at a dose of 5 Mrad with a cobalt-60 source. To induce the formation of acetal-like groups susceptible to hydrolysis within alginites, both the original high MW MVG and irradiated low MW MVG were oxidized with sodium periodate (NaIO₄) both at 0.1 M NaNO₃ and a right-angle laser light scattering detector (RALLS). A 0.1 M NaNO₃ buffer solution (pH 6.3) was used as a mobile phase, and alginites were dissolved in the mobile phase. Polymers separated through a set of two TSK-gel columns (G4000PWXL and G3000PWXL) were analyzed. From the weight-average molecular weight (Mₐ) and intrinsic viscosity [η] measured with the size exclusion chromatography system, Kuhn’s segment length (bₒ) was calculated, following the procedure proposed by Bohdanecký. The ratio between mannuronic acids and guluronic acids (M/G) of alginate was determined with a circular dichroism (CD) spectrometer (AVIV 202) and ¹H NMR (nuclear magnetic resonance) spectrometer. The CD spectra were acquired by measuring the absorbance of the solution (concentration ~ 0.4 mg/mL) at wavelengths from 250 to 190 nm at 25 °C. From the CD spectra, molar ellipticity values were acquired in units of deg·cm²/dmol. The M/G ratio was calculated by dividing the height of the peak appearing at 200 nm by that of the trough appearing at 220 nm. NMR spectra of solution prepared with D₂O were recorded with a 400 MHz NMR spectrometer (Varian Inova) at a temperature of 25 °C, a sweep width of 6000.6 Hz, a 35° pulse, and an acquisition time of 5.3 s. Integration of the peak at 5.0 ppm, which corresponds to the proton of guluronic acids, and that of the peak at 4.5 ppm, which corresponds to the proton of mannuronic acids, were compared.

The changes in the MW of oxidized alginites dissolved in DMEM and maintained at 37 °C were also monitored with size-exclusion chromatography on a weekly basis. This test was performed with a single sample for each condition. The degradation rate was calculated assuming the first-order degradation kinetics accepted for the random hydrolysis of polymers. Assuming that the total MW of alginate is larger than the MW of the repeating unit (m), the degradation rate constant k was calculated as follows.

\[
\frac{1}{\text{MW}_t} = \frac{1}{\text{MW}_0} + \frac{k}{m} t
\]

where MWᵣ is the MW of alginate at a time point t, and MW₀ is the initial MW of alginites.

Preparation and Characterization of Alginate Hydrogels. Calcium cross-linked alginate hydrogels were prepared by mixing 3% (w/w) alginate solutions with calcium sulfate (CaSO₄, Sigma) slurries. In preparing binary oxidized hydrogels, oxidized low and high MW MVG solutions were thoroughly mixed at a given ratio prior to mixing with calcium. The fraction of oxidized low MW MVG as a function of total alginate mass was varied from 0 to 0.75. The molar ratio between calcium and alginate sugar residues was kept constant at 1.0:0.3. The mixtures were immediately cast after mixing between glass plates separated with 2 mm spacers, and after 2 h the gels were cut into disks (24 mm diameter). The gels were stored at 37 °C in DMEM for 1 day before testing.

Covalently cross-linked gels were prepared by sequentially mixing 2% (w/w) alginate solutions prepared with 2-[N-morpholino]ethanesulfonic acid hydrate (MES, Sigma) buffer (at pH 6.5) with 1-hydroxybenzotriazole (HOBr, Aldrich), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC, Sigma), and adipic acid hydrazide (AAD, Sigma). The molar ratio between AAD and alginate sugar residues was kept constant at 1.0:0.15. After casting of the mixtures and cutting into disks, the gels were also incubated at 37 °C in DMEM.

The viscosity of pre-gelled solutions was measured using a controlled-stress rheometer (Bohlin Instrument) at 25 °C. Prior to the measurement, all samples were presheared at a high shear rate followed by rest for 5 min. While the shear stress was increased from 0.5 to 50 Pa, the resulting strains were measured, and the corresponding viscosity was calculated. From the shear-thinning curves, the low shear viscosities independent of the shear stress were compared, as they reflect the interactions between molecules at a low disturbance.

The elastic moduli (E) of the gels were measured by compressing at a constant deformation rate of 1 mm/s with a mechanical tester (MTS Bionix 100, MTS systems) at 25 °C. From the strain limited to the first 10% and resulting stresses (σ), the compressive elastic moduli of the hydrogels were calculated. Assuming that the alginate hydrogels fit an affine network model, the shear modulus (S) was obtained from the slope σ vs (λ - λ⁻¹) plot, where λ is the ratio of the deformed length to the undeformed length of the hydrogel.

The swelling ratios of the gels at equilibrium were also determined by measuring the weight of swollen gels following incubation at 37 °C for 24 h and the weight of dried alginate in each gel. The degree of swelling (Q) was defined...
as the reciprocal of the volume fraction of a polymer in a hydrogel ($v_2$):

$$Q = \frac{1}{v_2} = \left[ \frac{1}{\rho_p} \left( \frac{Q_m}{\rho_s} + \frac{1}{\rho_p} \right) \right]^{-1}$$  \hspace{1cm} (2)

where $\rho_p$ is the polymer density (0.8755 g cm$^{-3}$), $\rho_s$ 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 $S$ and $Q$, the effective number of cross-links ($N$) was determined,\(^{15}\) based on the rubber elasticity theory:

$$N = \frac{SQ^{-1/3}}{RT}$$  \hspace{1cm} (3)

where $R$ is the gas constant (8.314 J mol$^{-1}$ K$^{-1}$) and $T$ is the temperature at which the modulus was measured.

To evaluate degradation rate of the gels, changes in $E$ and $Q$ of the gels were monitored on a weekly basis. The medium was also exchanged on a weekly basis ($n = 4$ time point/condition).

**Transplantation of Bone Marrow Stromal Cells (BMSCs).** Primary bone marrow stromal cells were isolated from rat limbs following the procedure described elsewhere.\(^{16}\) The isolated cells were cultured through several passages prior to use, and cells between passages 5 and 6 were used in all experiments. To induce specific interactions between alginate gels and encapsulated BMSCs, both oxidized low MW and high MW MVGs were conjugated with oligopeptides of a sequence of (glycine)$_4$-arginine-glycine-aspartic acid-tyrosine (GGGGGRGDY, Commonwealth Biotechnology Inc.) following the procedure described elsewhere.\(^{17}\) The molar ratio of oligopeptides to alginate sugar residues was kept constant at 0.003:1. The solutions of alginate conjugated with oligopeptide were prepared with minimum essential medium (α-MEM, Gibco) supplemented with 10% bovine serum albumin (Gibco) and 1% penicillin/streptomycin (Gibco). To direct the differentiation of BMSCs to osteoblasts, bone morphogenic proteins (BMP-2, Chemicon) and recombinant human transforming growth factor (TGF-β3, Chemicon) were preincorporated in the alginate solutions at a density of 1 µg mL$^{-1}$.\(^{18}\) The BMSCs were mixed with solutions of nonoxidized high MW MVG and oxidized binary alginates to yield a density of $20 \times 10^6$ cells mL$^{-1}$. The weight fraction of oxidized low MW MVG was 0.75 in these experiments. The cell–alginate mixtures were mixed with CaSO$_4$ aqueous slurries to form the gels, and 200 µL of gel/cell constructs were injected subcutaneously into the back of anesthetized CB-17 SCID mice (Taconic Farmers Inc., $n = 4$ time point/condition). The mice were sacrificed after 24 weeks, and the injected samples were retrieved. The explants were fixed in 10% buffered formalin, dehydrated in ethanol, and embedded in paraffin. Sectioned samples were stained with Hematoxylin and Eosin and Goldner’s Trichrome for microscopic observation.

**Results**

**Chemical Modification of Alginates.** The molecular weight (MW) and chemical structure of alginate molecules were modified through $\gamma$-irradiation and oxidation (Figure 1).
Table 1. Number-Average Molecular Weight (MWn), Weight-Average Molecular Weight (MWw), Polydispersity, Kuhn’s Segmental Length (bK) of Alginates, and Fraction of Guluronic Acids (FG) Altered by γ-Irradiation and Oxidation

<table>
<thead>
<tr>
<th>Alginate</th>
<th>θ</th>
<th>MWn (g/mol)</th>
<th>MWw (g/mol)</th>
<th>Polydispersity</th>
<th>bk (nm)</th>
<th>FG</th>
</tr>
</thead>
<tbody>
<tr>
<td>High MW</td>
<td>0</td>
<td>220,000</td>
<td>270,000</td>
<td>1.2</td>
<td>14</td>
<td>0.6</td>
</tr>
<tr>
<td>MVG 0.01</td>
<td>70,000</td>
<td>120,000</td>
<td>1.7</td>
<td>11</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>40,000</td>
<td>70,000</td>
<td>1.8</td>
<td>6</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Low MW</td>
<td>0</td>
<td>40,000</td>
<td>60,000</td>
<td>1.5</td>
<td>22</td>
<td>0.6</td>
</tr>
<tr>
<td>MVG 0.01</td>
<td>50,000</td>
<td>60,000</td>
<td>1.2</td>
<td>19</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>30,000</td>
<td>40,000</td>
<td>1.1</td>
<td>7</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

θ is defined as the molar ratio between NaIO4 added to the reaction and the number of alginate sugar residues in the solution.

The original polymer had a weight-average MW (MWw) of 260,000 g/mol. Irradiating this high MW MVG at a dose of 5 Mrad decreased the MWw by 80% (Table 1). However, the irradiation increased Kuhn’s segmental length (bk) of the resultant low MW alginates. The bk of high MW alginates (14 nm) is in good agreement with the bk reported previously (15.5 nm).

Oxidation of alginates to cleave the carbon-—carbon bond of the cis-diol groups within saccharide rings also decreased the total MW of alginates in proportion to the molar ratio between NaIO4 added to the reaction and the number of alginate sugar residues in the solution (θ) (Table 1). Interestingly, the decrease in total MW of high MW MVG was more severe than the change in the low MW MVG. However, the MWw of oxidized high MW MVG was still 2-fold higher than the oxidized low MW MVG. The oxidation also reduced bk of both low and high MW MVG in proportion to θ.

Unlike the significant changes in MW and bk, the fraction of guluronic acids (FG) was not altered when polymers were irradiated and oxidized, as illustrated with circular dichroism (CD) spectra (Table 1). The independence of FG on modification of alginates was also supported with NMR spectra, which showed no change in the integration ratio between the proton peaks at 5.0 and 4.5 ppm.

The process of hydrolytic chain scission in the oxidized alginates following incubation in aqueous medium was examined by monitoring changes in the MW of alginates over time. Previously, we reported that the reduction in the MW of oxidized high MW alginates over time mostly occurred within the first 2 weeks. The low MW MVG, oxidized to the same θ, also demonstrated a rapid decrease in the MW within the same time period, followed by a gradual reduction for the following 2 weeks. The MW of oxidized alginates that degraded for 1 month agreed well with the theoretical MW for these polymers (based on the number of potential degradation sites/chain created by oxidation). The theoretical MW of completely degraded alginate was calculated using the target values of θ (i.e., 21,000 g/mol for oxidized high MW alginates and 20,000 g/mol for oxidized low MW alginates). A plot of measured 1/MW of oxidized alginates versus incubation time was linear, and the degradation rate constants k were calculated following eq 1 (Figure 2).

These data suggest one can readily modulate the degradation rate of the gels by mixing oxidized low and high MW MVGs at different weight fractions.

Physical Properties of Oxidized Gels. The elastic moduli (E) of alginates are highly dependent on the length of G blocks, while the viscosity of the alginate solutions is mainly dependent on the MW distribution of alginates or chains in solution. Oxidizing high MW MVG (θ = 0.01), which resulted in a change in total MW, led to a decrease in the low-shear viscosity (ηθ) of pre-gelled solution from 5.0 to 1.3 Pa s. In contrast, the E of the calcium cross-linked gels prepared with these solutions was only slightly decreased, from 136 kPa to 120 ± 7 kPa, as compared to the gels formed with nonoxidized high MW MVG chains (Table 2).

The effects of molecular weight distribution (MWD) of polymers on ηθ and E were also investigated with the binary gels by mixing, at various ratios, low and high MW MVGs oxidized at θ of 0.01 (Table 2). Increasing the fraction of oxidized low MW MVG (W(L)), at a given alginate concentration [3% (w/w)], exponentially decreased ηθ, as described with the expression ηθ ∝ θ−4W(L)). In addition, the dependency of ηθ on the shear stress also decreased with increasing W(L) (Figure 3). In contrast, increasing W(L) in the calcium cross-linked binary gels from 0 to 0.25 slightly increased E from 120 ± 7 kPa to 130 ± 3 kPa, which is almost equivalent to the E of the nonoxidized high MW MVG gels. Further increasing W(L) to values greater than 0.50 decreased E. However, the reduction in E after this point was much less than the great reduction in ηθ. No significant change in the swelling ratio (Qw) was observed. The number of cross-links (N0), calculated using eq 3, was slightly reduced by increasing W(L) from 0 to 0.50. Increasing θ of low MW MVG to 0.03 greatly decreased E, as its value went from 104 to 18 kPa, at a given W(L) of 0.50. Altogether, these results demonstrate that one can alter the viscosity of these pre-gelled solutions without severely deteriorating the mechanical rigidity of the post-gels by adjusting the MWD of alginates under certain conditions.

The degradation behavior of oxidized binary hydrogels having different W(L) values was examined by monitoring changes in the E and Q values of the gels over time. As a control, nonoxidized high MW MVG hydrogels showed a gradual decrease in E over 1000 h, following incubation in the conventional medium (DMEM) used for cell culture.
exhibited a gradual reduction in (Figure 4a). Oxidized high MW MVG gels (0.05) showed a rapid decrease in $E$ between 200 and 500 h, leading to a reduction in $E$ by 70% (Figure 4a). The time period in which $E$ rapidly degraded agreed well with the time period noted to correspond to a rapid reduction in MW of oxidized alginites. Incubating gels in calcium-free DMEM also accelerated the decreases in $E$ of the gels (Figure 4b). These results suggest the slower reduction in $E$ of these gels after 500 h in regular DMEM was attributed to a balance between dissociation and reassociation of polymer chains and Ca$^{2+}$ present in DMEM. However, in either case, the rate of decrease in $E$ of the gels could be regulated with the MWD of polymers.

Changes in the number of cross-links ($N$) in the gels was calculated, following eq 3, and increasing W(L) greatly accelerated the decrease in $N$ (Figure 5), as expected. The degradation kinetics could be represented by an exponential regression fit (eq 4), as follows.

\[ \frac{N}{N_0} = \exp(-k't) \]  

Increasing W(L) to 0.75 significantly raised the degradation rate constant ($k'$), as compared to the nonoxidized gels and oxidized high MW MVG gels (Table 2). The incubation condition [±Ca$^{2+}$] changed $k'$ at a given W(L), but increasing W(L) led to a higher value of $k'$ in both cases.

To ensure that the MWD of polymers alters the gel degradation, irrespective of the cross-linking type, we also examined the degradation behavior of covalently cross-linked

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Table 2. Physical Properties of Ca$^{2+}$ Cross-Linked Hydrogels Having Different Weight Fractions of Oxidized Low MW MVG [W(L)]$^a$

<table>
<thead>
<tr>
<th>$\theta$</th>
<th>W(L)</th>
<th>$\eta_0$ (P)</th>
<th>$E_{Ca}$ (kPa)</th>
<th>$Q_{Ca}$</th>
<th>$N_{Ca}$ ($\times 10^{-7}$ mol/cm$^3$)</th>
<th>$k_1'_{Ca}$ (x $10^{-4}$ h$^{-1}$)</th>
<th>$k_2'_{Ca}$ (x $10^{-4}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>50</td>
<td>136</td>
<td>21 ± 0.9</td>
<td>64 ± 0.6</td>
<td>8 ± 0.5</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>0.01</td>
<td>0</td>
<td>13</td>
<td>120 ± 7</td>
<td>24 ± 0.8</td>
<td>58 ± 3</td>
<td>14 ± 1</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>0.25</td>
<td>7</td>
<td>130 ± 3</td>
<td>53 ± 0.6</td>
<td>64 ± 0</td>
<td>17 ± 1</td>
<td>29 ± 1</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>0.50</td>
<td>2.5</td>
<td>104</td>
<td>24 ± 0.5</td>
<td>50 ± 0</td>
<td>17 ± 1</td>
<td>17 ± 1</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>0.75</td>
<td>0.8</td>
<td>76 ± 3</td>
<td>21 ± 1</td>
<td>34 ± 2.7</td>
<td>29 ± 1</td>
<td>39 ± 4</td>
<td>42 ± 5</td>
</tr>
</tbody>
</table>

$^a$ $\eta_0$ is the low shear viscosity of 3% (w/w) pre-gelled solution. $E_{Ca}$, $Q_{Ca}$, and $N_{Ca}$ represent the elastic modulus, swelling ratio, and number of cross-links for the calcium cross-linked gels, respectively. $k_1'$ is the degradation rate constant of the gel incubated in regular DMEM, and $k_2'$ is that of the gel incubated in Ca$^{2+}$-free DMEM. $\theta$ is defined as the molar ratio between NaIO$_4$ added to the reaction and the number of alginate sugar residues in the solution.
hydrogels consisting of different W(L) values. As expected, a bimodal MWD of polymers was effective in regulating the degradation of the gels cross-linked with adipic acid dihydrazide (AAD), which are more stable than the Ca\(^{2+}\)-cross-linked gels in physiological condition. The $E$, $Q$, and $N_0$ of the covalently cross-linked gels were less dependent on W(L), as compared with Ca\(^{2+}\) cross-linked gels (Table 3), as one would expect. However, $N$ of the binary oxidized gels gradually decreased over 1000 h (Figure 6), leading to a higher value of $k'_{\text{AAD}}$ (calculated from eq 4) under this condition than that calculated for the oxidized high MW gels (Table 3). These results confirm that adjusting the MWD mediates the degree and rate of gel degradation, while limiting the changes in $N_0$, irrespective of the mechanism of cross-linking.

**Engineering of Bone Tissues.** The relevance of controlling gel degradation with this system was evaluated in the context of new bone formation via transplantation of bone marrow stromal cells (BMSCs). Tissues engineered using the slowly degrading nonoxidized high MW MVG gel or the rapidly degrading oxidized binary gel [W(L) = 0.75] formed from cross-linking with calcium were compared.

Both types of gel constructs maintained their structure over 6 months following injection into mice. However, examination of cross sections of tissue explants revealed a great difference in the cellularity and quality of bone tissue depending on which gel was used for cell transplantation. Use of the nonoxidized high MW MVG gels resulted in large areas of residual gel, and the formation of new bone tissue was limited (Figure 7a). In contrast, the binary oxidized gels degraded more rapidly, as indicated by the presence of only small gel fragments. This appeared to facilitate the formation of bone tissue, as illustrated with more cells, matrix, and tissue resembling bone in tissue sections from this condition (Figure 7b). More abundant blood vessels were also observed within these tissues (Figure 7b). The quality of the engineered tissues was also evaluated with the specific staining of explant sections with Goldner’s Trichrome to specifically highlight bone tissue.\(^{23}\)

The deposition of collagen was highly limited within the slowly degrading high MW MVG gels (Figure 7c), while large quantities of deposited collagen were observed within the tissue constructs formed using the binary oxidized gels (Figure 7d).

**Discussion**

This study demonstrates a novel method to control the physical properties, including the elastic moduli and degradation rate of gels, and the importance of this approach in the quality of bone tissue formed from transplanted bone marrow stromal cells (BMSCs). Adjustment of the molecular weight distribution (MWD) of alginates oxidized under conditions that maintain their gel-forming ability and chain rigidity allowed us to regulate the degradation rate of the gels while limiting changes in the initial physical properties of the gel (e.g., elastic modulus and swelling ratio).

The molecular weight (MW) and chemical structure of alginate were modified via $\gamma$-irradiation and oxidation reaction. The increase in Kuhn’s segment length ($b_0$) with no change in the ratio between guluronic acids and mannuronic acids ($F_G$) upon irradiation, despite the decrease in total MW of the polymer chains, indicated that there was a selective...
chain breakage at the most flexible blocks (i.e., alternating mannuronic acid and guluronic acid blocks) at a given irradiation dose, as schematically described in Figure 1. The length of G blocks, which are the most inflexible blocks in alginate, were likely not decreased. Decreases in $b_k$ upon oxidation verify the opening of saccharide rings. However, the finding that $F_G$ did not change as a result of this treatment, despite the reduction in MW, suggested that most chain breakages and opening of sugar rings occurred at the most flexible blocks of the polymer chains. The increased chain breakages within the high MW alginate, as compared with irradiated low MW MVG, are attributed to the higher fraction of more flexible blocks in these alginate chains which may be more susceptible to chain breakage by oxidants.

Decreases in the polymer MW upon irradiation and oxidation significantly reduced the viscosity of pre-gelled solutions, due to the weaker physical interactions between the smaller polymers. In addition, similar to the original alginate solution, the viscosity of oxidized alginate solution was independent of time, which indicates the absence of physically associated structures which could potentially form due to reaction of the aldehydes formed from oxidation. However, the decrease in MW did not lead to a significant reduction in the elastic moduli of gels, when the polymers irradiated at 5 Mrad were oxidized at $\theta = 0.01$. This lesser dependence of mechanical rigidity than viscosity on the MW of the polymer is likely due to the insignificant changes in the length of G blocks in polymer chains at this value of $\theta$, as deduced from the small reduction in $b_k$. The G block length largely determines the elastic modulus of calcium cross-linked or covalently cross-linked gels. In contrast, the significant drop in the elastic moduli of the gels as $\theta$ exceeded 0.03 likely resulted from the oxidation of G blocks. Together, these data indicate values of irradiation intensity and degree of oxidation that do not damage the gel-forming ability of alginates, while decreasing the length of the polymer chains.

The decrease in MW of the partially oxidized alginates over time in aqueous solutions followed first order degradation kinetics, and confirmed that hydrolytic chain breakage was occurring at the opened uronic acid residues. This result is similar to that observed with hydrolytically labile cellulose or κ-carrageenan. Interestingly, the initial MW of the alginates regulated the degradation rate of the oxidized alginates, likely due to the increased hydrolyzable surface area of low MW MVG.

Chain scission in the oxidized high MW MVG failed to cause significant changes in gel degradation properties, as compared to nonoxidized high MW gels maintained under similar conditions. This result implies that the chain breakages occurring in the partially oxidized high MW MVG were not sufficient to cause complete separation between the cross-linking junctions (Figure 8b) that regulate the elastic response and hydration of gels. In contrast, gel degradation was facilitated with the incorporation of oxidized low MW MVG, despite little differences in the initial physical properties and number of cross-links of the gels. In contrast, incorporation of nonoxidized low MW MVG, instead of the oxidized low MW alginate, in the binary gels increased the stability of the gels. This finding is likely related to the high mobility of the low MW MVG, which may enhance the reassociation of polymer chains which dissociate due to ion exchange. Therefore, the faster degradation in the binary oxidized system likely results from an increased separation between cross-linked domains upon chain breakage of low MW MVG (Figure 8a), coupled with the faster chain breakage of the low MW MVG. Hence, we propose that adjusting MWD of alginates at various oxidation degrees would allow us to regulate the degradation rate of the gels over a broad range. Effects of the MW of hydrolytically labile polymers on their degradation rate have been reported with poly(lactic acid). However, the present study further demonstrates that the degradation and mechanical properties of gels can be regulated in a refined manner by utilizing a bimodal MWD of the polymer.

The oxidized binary hydrogels improved the formation of bone tissue from transplanted cells, as compared to non-degrading gels. This confirms the importance of the degradation rate of gels used in engineering new tissues, as previously demonstrated with the low MW or highly oxidized alginate gels. In this system, BMSCs encapsulated in the gel were likely stimulated down the osteogene pathway by two growth factors also encapsulated in the gel. The subsequent deposition of bone tissues within the gel constructs, as well as cell proliferation, was facilitated by the gradual degradation of the gels. Simultaneously, BMSCs within the binary gels likely secreted angiogenic factors to stimulate the endothelial cells neighboring the gel construct, and which led to infiltration of blood vessels into the gel construct. It is also possible that host cells recruited to the
gel, by release of the growth factors, participated in bone formation. To further enhance the quality of engineered bone tissues formed from BMSCs with this system, one may optimize the release rates of supplemental growth factors incorporated in the gels. In addition, the use of other growth factors that drive vascularization within the gels [e.g., vascular endothelial growth factor (VEGF)] may also improve the quality of the engineered bone tissues.

In conclusion, controlling the MWD of properly tailored polymers allows one to regulate mechanical rigidity and degradation of gels in a sophisticated manner, and enables the development of cell transplantation vehicles desirable for engineering bone tissue. This material design approach may also be useful in the design of vehicles to deliver bioactive macromolecules and engineer other tissues. This approach may be useful for manipulating the physical properties of a broad array of other polymeric materials and hydrogels.

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References and Notes

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