Rigidity of Two-Component Hydrogels Prepared from Alginate and Poly(ethylene glycol)−Diamines

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Received April 6, 1999; Revised Manuscript Received June 18, 1999

ABSTRACT: Alginate hydrogels have been attractive for a variety of biomedical applications, but they possess limited mechanical properties when ionically cross-linked with divalent cations. Therefore, covalent cross-linking of alginate with poly(ethylene glycol)−diamines of various molecular weights was investigated as a means to generate hydrogels with a range of mechanical properties. Hydrogels with a range of elastic moduli could be generated by controlling either the chain length of the cross-linking molecule or the cross-linking density. The elastic modulus increased gradually with an increase in cross-linking density or weight fraction of PEG in the hydrogel up to ∼27% (w/w) of PEG. The change of mechanical properties was interpreted in terms of molecular weight between cross-links (M_c) according to the rubber-elasticity model, and the results of this analysis were generally consistent with the measured PEG−diamine incorporation efficiencies in this range. However, as the weight fraction of PEG in the hydrogels increased above 27%, regardless of the molecular weight of PEG, the elastic moduli decreased. This is not consistent with prediction based on the rubber-elasticity theory, and this is likely due to the fact that this model does not consider cross-linking with a second macromolecule. Importantly, the results of this study suggest that the mechanical properties of hydrogels will be strongly affected by the properties of the cross-linking molecule as the M_c of hydrogels falls below the molecular weight of the cross-linking molecule.

Introduction

Hydrogels belong to a class of polymers that can swell to a large extent in water by maintaining their three-dimensional network structure in the swollen state. Although the first report on the use of hydrogels for biomedical applications is almost 40 years old,[1] the interest for these materials is still growing. Cumulative evidence has shown that hydrogels can be highly biocompatible due to a unique combination of properties that include low interfacial tension with surrounding biological fluids and tissues,[1,2] high water content,[3] and soft and rubbery consistency.[4] Over the past years hydrogels have been used in a number of biomedical applications such as carriers for the controlled release of drugs and bioactive macromolecules.[5,6]

A relatively new application of hydrogels is to serve as a delivery vehicle for cells in a variety of tissue engineering applications.[7,8] The reconstruction of tissues and organs, utilizing polymeric materials that mimic the properties and functions of the extracellular matrix, offers an attractive alternative for the treatment of patients suffering tissue failure or loss. One tissue engineering approach includes isolation of cells from a small tissue sample and its expansion in vitro to generate a large cell mass. The cells are then seeded onto a suitable polymer matrix and transplanted into a patient where a new tissue is formed and structurally integrated with the natural tissue.[7] Hydrogels are attractive materials for soft tissue engineering applications due to their high similarity to the body's own highly hydrated compositon.[9−11] They can serve as cell delivery vehicles and guide and support the formation of the new tissue in vivo.[7,12] The mechanical properties (i.e., tensile or compressional modulus) of swollen hydrogels in these and other biomedical applications are important because they must possess mechanical strength and flexibility sufficient to withstand compressional forces from the surrounding tissues in vivo without deformation or collapse.[13,14] In addition, it is of great interest to create hydrogels with controlled mechanical properties since the mechanical properties of materials to which cells are adherent can profoundly affect the function of the cells.[15]

Sodium alginate is a particularly attractive material to form hydrogels for biomedical applications.[16,17] It is a naturally derived linear polysaccharide comprised of β-D-mannuronic acid (M-block) and α-L-guluronic acid (G-block) units arranged in blocks rich in G units or M units, separated by blocks of alternating G and M units. Alginate is considered biocompatible and forms hydrogels in the presence of multivalent cations, (e.g., Ca^{2+}) through the ionic interaction between the carboxylic acid group located on the polymer backbone and the cation.[18] Ionically cross-linked alginate gels possess a range of mechanical properties, which depends on the nature of the alginate (M:G ratio) and the stoichiometry of the alginate with the chelating cation. The main contribution to structural integrity comes from the association of G-block sequences with the divalent cations. Conversely, the physical properties (i.e., mechanical strength) of ionically cross-linked alginate gels vary over time due to an exchange of the binding cations with other available monovalent cations.[19] The uncontrollable disintegration of ionically cross-linked alginate hydrogels, along with their limited range of mechanical properties, presents significant limitations to the biomedical applications of this material.

This paper describes the preparation of alginate hydrogels with controllable mechanical properties in the

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10.1021/ma990514m CCC: $18.00
Published on Web 08/07/1999

swollen state. We covalently cross-linked alginate with several poly(ethylene glycol) (PEG) derivatives. PEG was chosen as the cross-linking molecule on the basis of properties including its biocompatibility and high hydrophilicity.[20] It is soluble in organic and aqueous solutions, commercially available at various molecular weights, and can be easily chemically modified.[20,21] Furthermore, it has been shown that proteins which were modified with PEG showed decreased immunogenicity and antigenicity at an increased circulation time in the body.[22,23] The hydrogels obtained from alginate and PEG are composed of two polymers with different mechanical properties. The flexible PEG chains are thought to provide elasticity to the system while the stiff alginate chains provide mechanical strength to this two-component system. Multicomponent system can offer versatility to the hydrogel in terms of degradation and mechanical properties. It is expected that the properties of such a system can be varied by changing its composition, the length of the cross-linking molecule, or the cross-linking density as desired.

Experimental Section

Materials. Sodium alginate was purchased from Pronova Biopolymers Inc. (Porthsmouth, NH) and had an overall guluronic acid (G-block) content of approximately 70% as reported by the manufacturer. Poly(ethylene glycol) (PEG) with number-average molecular weight of 200, 400, and 1000 were purchased from Lancaster Synthesis Inc. (Windham, NH), and that of 3400 was purchased from Aldrich (Milwaukee, WI). PEGs were dried for 24 h over phosphorus pentoxide in a vacuum before use. *N*-*tert*-Butoxycarbonylglycine, ammonia-free glycine, ninhydrin reagent, and morpholinoethanesulfonic acid (MES) were purchased from Sigma (St. Louis, MO); 4-(dimethylamino)pyridine (DMAP), dicyclohexylcarbodiimide (DCC), 1-ethyl-3-(dimethyl aminopropyl)carbodiimide (EDC), and *N*-hydroxysuccinimide (NHS) were purchased from Aldrich (Milwaukee, WI). All chemicals were used as received.

Methods. GPC measurements were performed at room temperature using a system equipped with an isocratic pump (P1000, Thermo Separation Products) and a triple detector system (Viscotek) including a laser refractometer (LR 40) and a dual detector (T60, differential viscometer and RALLS). A 0.1 M $NaNO_3$ buffer solution (pH 6.3) was used as a mobile phase, and the flow rate was 0.7 mL/min. A set of two TSK-gel columns ($G4000PW_{XL}$ and $G3000PW_{XL}$) was used. The samples were dissolved in the mobile phase, filtered, and injected through a Rheodyne valve (model 7010) equipped with a 100 μL injection loop. The number-average molecular weight (M_n) of alginate was 1.7×10^5 with a polydispersity index (PDI) of 2.3. FT-IR spectra were recorded using an Avatar 360 spectrophotometer (Nicolet) using sodium chloride crystals. ^{1}H NMR was performed on a Bruker AM (360 MHz). Spectrophotometric measurements were determined using a Perkin-Elmer Lambda 12 UV/vis spectrophotometer. The elastic modulus of the alginate hydrogels was determined at room temperature by compression measurements on a MTS Bionix 100 (load cell = 10 N) with a cross-head displacement rate of 1 mm/min. The Young's modulus (E) was calculated from the slope of the initial linear region of a plot of stress (σ) versus strain (ϵ).

Synthesis of Poly(ethylene glycol)−Diamines. Diamino-terminated cross-linking molecules with different molecular weights were synthesized in a two-step reaction following a general procedure[24] utilizing standard carbodiimide chemistry as outlined in Figure 1. All PEGs were dried via azeotropic distillation in toluene, and the solvent was then removed in a vacuum. PEG (**1**, 2.5 mmol) with various molecular weights was reacted with N-*t*-Boc-glycine (**2**, 5.5 mmol) and DMAP (1.25 mmol) in minimal amount of CH_2Cl_2. Subsequently, 6 mmol of DCC was added, and the reaction mixture was stirred at 0 °C for 24 h. The dicyclohexylurea (DCU) was filtered, and

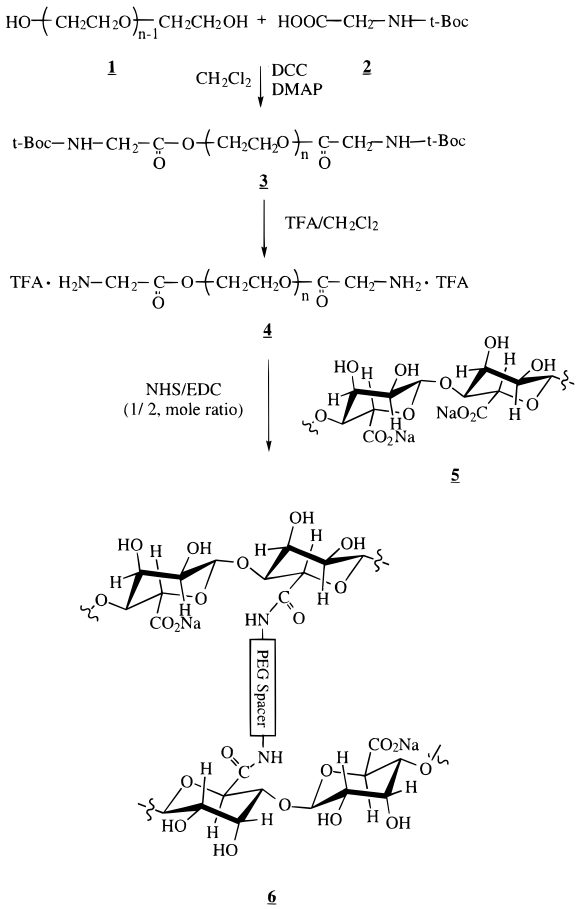

Figure 1. Reaction scheme for the synthesis of PEG−diamine and subsequent cross-linking reaction of sodium alginate.

the filtrate was concentrated in a vacuum at room temperature. The resultant was dissolved in minimal amount of acetone and cooled overnight, and the precipitated DCU was filtered off. For the removal of a *t*-Boc group, PEG-*t*-Boc-amino acid diester (**3**) was dissolved in a mixture of CH_2Cl_2/TFA (1/1, v/v). The reaction mixture was stirred for 3 h and then evaporated to dryness. The deprotected derivative (**4**) was dissolved in a 15% NaCl solution, and the pH was adjusted to 5. The solution was filtered to remove water insoluble *tert*-butyl salts. The filtrate was extracted three times with chloroform; the organic phases were combined and dried over Na_2SO_4. The Na_2SO_4 was filtered, the solvent was evaporated, and the oily residue was dried under vacuum over P_2O_5 at room temperature for 2 days. In the case of PEG of molecular weight of 3400, the derivative was dissolved in a small amount of CH_2Cl_2 and precipitated by ether. The polymer was collected, dissolved in ethanol, and reprecipitated by ether. TLC was carried out for all PEG−diamines on silica gel N-HR UV 254 using 1-butanol/acetic acid/water (4/1/1, v/v/v) as an eluent and ninhydrin to visualize the chromatogram. The structure and the completeness of deprotection were verified by ^{1}H NMR and FT-IR. The disappearance of the chemical shift at $\delta = 1.4$ ppm in the NMR spectra suggested the complete removal of the *t*-Boc group. Throughout this paper the cross-linking molecules are denoted as P200, P400, P1000, and P3400 depending on the molecular weight of the starting PEG.

FT-IR (NaCl): 1757 (C═O), 1658 (NH_2), 1173 (CH_2OCH_2) cm^{-1}.

^{1}H NMR ($CDCl_3$): $\delta = 3.45$ (s, NC**H**$_2$CO), 3.6 (s, OC**H**$_2$C**H**$_2$), 4.4 (m, **H**NCH_2CO) ppm.

Covalently Cross-Linked Alginate Networks. Sodium alginate was covalently cross-linked with PEG−diamines having different molecular weights (Figure 1) using a previously described optimized set of conditions.[12] A 2% (w/w) sodium alginate solution was prepared in a buffer solution of

0.1 M MES and 0.5 M NaCl, and the pH was adjusted to 6. The viscous solution was filtered through a 0.45 μm filter to remove aggregates. The molar ratio of EDC:NHS:COO^- was 1:0.5:1. A 87.5 mg (0.76 mmol) sample of NHS and 291.4 mg (1.52 mmol) of EDC were added to 15 g of a 2% (w/w) alginate solution to activate the carboxylic acid groups on the polymer backbone. The solution was agitated to obtain a homogeneous solution followed by the addition of PEG−diamines. The solution was cast between glass plates separated by a spacer of 2 mm. After 24 h the gel was cut into disk with diameter of 12.7 mm and placed in double distilled water to remove unreacted material and low molecular weight byproducts.

Determination of Cross-Linking Efficiency. The ninhydrin assay was utilized to determine the extent of effective cross-linking (double-end anchorage), unreacted pendent amino groups during the coupling reaction (single-end anchorage), and unreacted cross-linking molecules. Briefly, the hydrogel was dried at room temperature until a constant weight was achieved and ground into small particles. A small amount of the dried hydrogel was suspended in 1 mL of 1 M acetate buffer (pH 5), and ninhydrin reagent was added. The mixture was kept in boiling water for 15 min. After incubation, 15 mL of an ethanol/water mixture (1/1, v/v) was added, and the reaction mixture was cooled to room temperature for 1 h in a dark place. Ninhydrin reacts with free amino groups and creates a blue water-soluble compound.[25] It was considered that the hydrolysis of the amide bond was negligible during this assay as the amide bond is generally considered stable at this experimental condition.[26,27] The amount of free amino groups in the hydrogel was spectrophotometrically determined by measuring the absorbance of the supernatant at 570 nm. Glycine (3−10 μmol) was used as a reference material. The number of unreacted amino groups was determined in the hydrogels both before and after they were leached in water. The number of amino groups found in the unleached hydrogels corresponds to single-end anchorage and unreacted ones (A). Subtraction of (A) from the total amount of added amino groups reveals the extent of the effective cross-linking reaction (B). The number of amino groups found in the leached hydrogels corresponds to single-end anchorage (C). Subtraction of (C) from (A) results in the amount of unreacted PEG−diamines that have not participated in the cross-linking reaction.

Equilibrium Swelling Properties. Hydrogels were placed in deionized water at 25 °C for 5 days, and the water was changed frequently. Assuming that the network swells uniformly in all directions, the equilibrium swelling ratio (Q) can be defined as a ratio of the weight of swollen gel to the weight of dry gel. The weight of the dry gels was determined by drying the gel disks in a vacuum at 80 °C to remove tightly bound water molecules from the polymer.

Results

In the first set of experiments, sodium alginate was covalently cross-linked with P200, P400, and P1000 at the theoretical cross-linking density of 15% to determine how cross-linking molecules with different molecular weights would affect the mechanical properties of the resulting hydrogel. The theoretical cross-linking density was calculated on the basis of the concentration of cross-linking molecule added to the alginate solution. The efficiency of each cross-linking reaction was determined spectrophotometrically by quantifying the amount of unreacted PEG−diamine (0.2−5.3%) and the amount of single-end reacted PEG−diamine (2.5−7.3%) (Table 1). The majority of amino groups were covalently bound to alginate with an overall cross-linking efficiency (inter- and intramolecular) of around 90% for all three kinds of PEG−diamines. The elastic moduli of the swollen hydrogels were determined next from stress−strain measurements and were found to depend on the chain length (number of repeat units) of the cross-linking molecules (Table 1). Sodium alginate cross-linked with P200 and P400 exhibited relatively low elastic moduli (9.7 ± 1.6 and 20.1 ± 1.1 kPa, respectively). Alginate cross-linked with P1000 exhibited a significant increase in elastic modulus (109.4 ± 9.5 kPa).

Table 1. Cross-Linking Efficiency, Elastic Modulus in Compression, and Molecular Weight between Cross-Links (M_c) of Alginate Hydrogels[a] Cross-Linked with Various Poly(ethylene glycol)−Diamines

cross-linker	cross-linking efficiency (%) double-end anchorage	single-end anchorage	unreacted	elastic modulus (kPa)	M_c[b] (g/mol)
P200	92.2	2.5	5.3	9.68 ± 1.6	16912
P400	90.0	6.2	3.8	20.1 ± 1.1	8747
P1000	92.5	7.3	0.2	109.4 ± 9.5	1463

[a] Hydrogels with theoretical cross-linking density of 15%. Theoretical cross-linking density was calculated based on a 2% (w/w) alginate solution and molecular weight of the repeat unit (M_0 = 198). [b] M_c was calculated from eq 1.

Next, the mechanical data were used to determine the molecular weight between cross-links M_c (Table 1). For affine deformation the shear modulus (G) can be obtained as the slope from a plot of σ versus $-(\lambda - \lambda^{-2})$, where λ is the ratio of the deformed length to the undeformed length of the hydrogel.[28] Over the range of strain covered, all plots were linear, and the ratio of E to G was approximately 3 in all cases. Therefore, it was assumed that the hydrogels were noncompressible materials, and the molecular weight between cross-links (M_c) was calculated according to eq 1.[28,29]

$$M_c = \frac{cRT}{G} \tag{1}$$

R is the gas constant (8.3143 J mol^{-1} K^{-1}), T is the temperature (298 K) at which the modulus was measured, and c is the concentration of alginate (g m^{-3}) in the cross-linking solution. The experimentally determined M_c's for the P200 and P400 cross-linked alginates showed a strong deviation from the theoretical value (1320 g/mol) calculated using the known amount of cross-linker added in the reaction. In contrast, the value for P1000 was in relatively good agreement with the theoretical value (Table 1).

In the second set of experiments, P1000 was used as a cross-linking molecule, and the theoretical cross-linking density was varied from 2 to 50% to determine the effect of cross-linking density on the mechanical properties of the hydrogels. The total number of incorporated amino groups (double-end and single-end reacted) was over 90% in most reaction conditions (data not shown). However, a significant number of cross-linking molecules reacted on one side only at the lowest cross-linking density (2%). Increasing the theoretical cross-linking density from 2 to 15% resulted in increasing elastic moduli of alginate hydrogels ranging from 32 to 110 kPa (Figure 2). However, further increasing the cross-linking density (30 and 50%) resulted in weaker hydrogels with moduli of 56 and 35 kPa, respectively.

To further investigate the unexpected behavior of the hydrogels cross-linked with P1000 at the higher cross-linking density, P3400 was used in the third set of experiments. Initially the modulus increased with an increase in cross-linking density as expected and reached a maximum (89.5 ± 6.4 kPa) at a cross-linking density of 5% (Figure 3). However, the moduli then declined

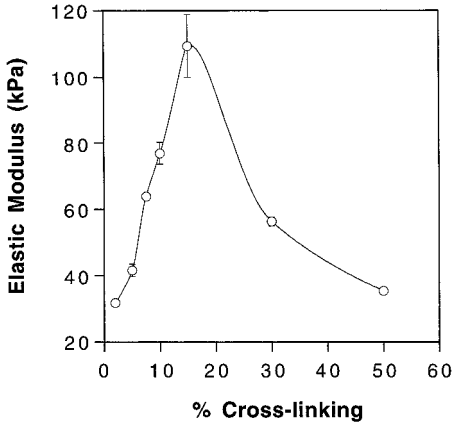

Figure 2. Elastic modulus in compression as a function of the theoretical cross-linking density utilizing P1000 as a cross-linking molecule. Values represent mean ($n = 5$) and standard deviation.

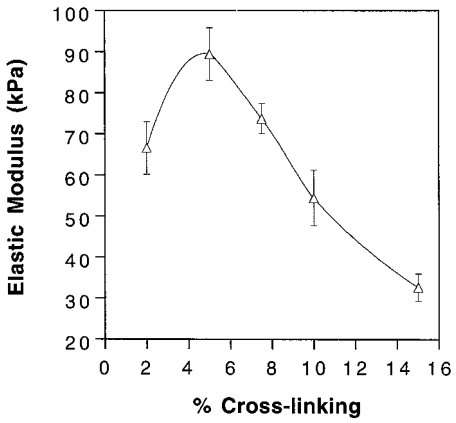

Figure 3. Elastic modulus in compression as a function of the theoretical cross-linking density utilizing P3400 as a cross-linking molecule. Values represent mean ($n = 5$) and standard deviation.

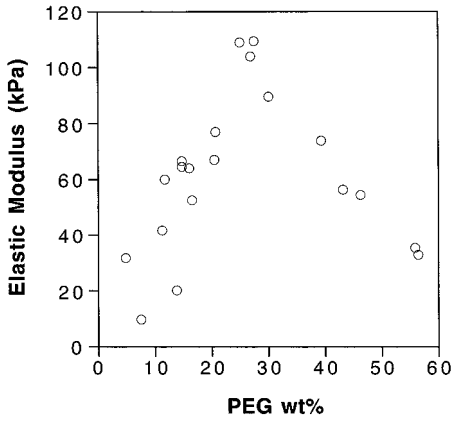

Figure 4. Effect of PEG weight fraction on the elastic modulus in compression of alginate hydrogels cross-linked with PEG−diamines of different molecular weights.

gradually despite an increase in PEG−diamine content added to the reaction. Summing all cross-linking data into one plot indicated a striking relation between elastic modulus and the weight fraction of the PEG in the hydrogels (Figure 4). There was a clear divergence in elastic modulus at PEG content of approximately 27 wt %. Hydrogels with PEG content up to that value followed the expected trend of an increase in modulus with an increase in cross-linking density. The molecular

Table 2. Theoretically and Experimentally Determined Molecular Weight between Cross-Links (M_c) and Equilibrium Swelling Ratio (Q) for Alginate Hydrogels Cross-Linked with P1000 or P3400

		P1000		P3400	
cross-linking density[a] (%)	theor M_c[b] (g/mol)	M_c (g/mol)	Q[c] (g/g)	M_c (g/mol)	Q[c] (g/g)
2	9900	4942	330 ± 27	2318	696 ± 25
5	3960	3689	223 ± 16	1710	224 ± 16
7.5	2640	2392	162 ± 19	1956	140 ± 4
10	1980	2019	103 ± 4	2800	197 ± 16
15	1320	1463	88 ± 2	4546	247 ± 10
30	660	2802	56 ± 10	n.a.[d]	n.a.[d]
50	396	4303	117 ± 6	n.a.[d]	n.a.[d]

[a] Cross-linking density was theoretically calculated based on a 2% (w/w) alginate solution and molecular weight of the repeat unit ($M_0 = 198$) and expressed as mol %. [b] The theoretical M_c was calculated from number-average molecular weight of alginate (1.7×10^5) and $M_0 = 198$ assuming complete intermolecular incorporation of the cross-linking molecule. [c] Mean ± SD ($n = 4$). [d] Not available.

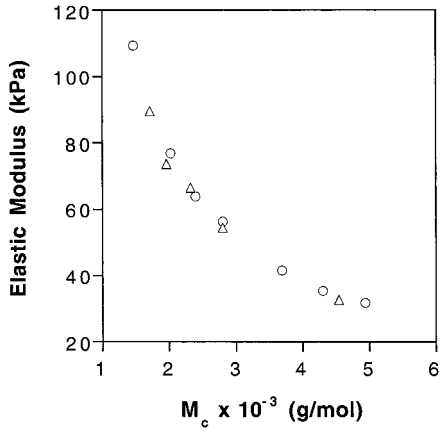

Figure 5. Elastic modulus in compression as a function of the experimentally determined molecular weight between cross-links (M_c) for P1000 (○) and P3400 (△).

weights between cross-links (M_c) were calculated and are listed in Table 2. They were in fair agreement with the theoretical M_c's in this range of added PEG. Hydrogels with PEG content higher than 27 wt % showed a decrease in modulus despite their higher cross-linking density, and their M_c's deviated strongly from the theoretical values. M_c also did not show a significant dependence on the specific cross-linking molecule used in this study. Hydrogels with nearly identical elastic modulus were obtained from both cross-linking molecules (P1000 and P3400) at different cross-linking densities (Figure 5). The equilibrium swelling ratio (Q) of the hydrogels cross-linked with P1000 and P3400 were also measured with respect to their cross-linking density (Table 2). The swelling ratio for both cross-linking molecules followed the same overall trend as observed in the elastic moduli. Initially, the equilibrium swelling ratio decreased with increasing cross-linking density and weight fraction of PEG and then increased.

Discussion

Hydrogels have been prepared by covalent cross-linking of alginate solutions with PEG−diamines. The reaction showed high efficiency, and hydrogels with widely varying mechanical properties could be formed with this approach. The structure and the corresponding mechanical properties of these hydrogels could be varied

by either changing the chain length of the cross-linking molecule or varying the cross-linking density. However, the mechanical properties were also strongly dependent on the weight fraction of PEG in the hydrogels, and samples with high PEG content exhibited a low elastic modulus despite their high cross-linking density. Swelling data followed a similar overall trend as observed in the elastic moduli experiments.

We have developed a series of covalently cross-linked alginates by utilizing standard carbodiimide chemistry with a high cross-linking efficiency. Other methods have been previously used to stabilize alginate by covalent cross-linking through the carboxylic group[30] or through the hydroxyl groups of the alginate.[31,32] However, few mechanical properties, swelling properties, or cross-linking efficiencies of these materials have been reported. In addition, all cross-linking molecules used in this study also possess an ester linkage which renders a hydrolytically labile linkage in the hydrogel. This is important for the intended application of these hydrogels as biodegradable scaffolds for tissue engineering.

Hydrogels prepared from covalently cross-linked alginate with P200 and P400 possessed elastic moduli much lower than those of hydrogels cross-linked with P1000 at the same cross-linking density. This was initially surprising considering the high incorporation of all three PEG−diamines into these hydrogels, which should result in a molecular weight between cross-links (M_c) much closer to the theoretical value (1320 g/mol). We thus hypothesize that the low moduli resulted from a large amount of intramolecular cross-links with the P200 and P400. Other studies have reported that flexible low molecular weight cross-linking molecules favor the formation of small loops and thus reduce intermolecular cross-linking efficiency.[33] The findings in our current study are in good agreement with this explanation. The experimentally determined M_c of hydrogels cross-linked with P1000 (1463 g/mol) was in good agreement with the theoretical value (1320 g/mol), suggesting that longer cross-linking molecules are more efficient in the formation of intermolecular cross-links.

Alginate hydrogels cross-linked with longer cross-linking molecules (P1000 and P3400) exhibited a surprising maximum in elastic moduli as the cross-linking density was increased. Hydrogels cross-linked with P1000 and P3400 showed an increase in modulus with increasing cross-linking density up to 15 and 5%, respectively. Hydrogels formed with both cross-linking molecules showed a decrease in M_c with increasing PEG content in this range, which is in accordance with the rubber-elasticity theory. However at higher cross-linking densities, M_c increases, indicating a decrease in the rigidity of the network. This occurs at a weight fraction of PEG of approximately 27% and corresponds to the situation where the theoretical M_c (calculated assuming complete intermolecular cross-linking by the PEG) becomes smaller than the molecular weight of the PEG molecule. This behavior is inconsistent with the theory of rubber elasticity, and we hypothesize that this results because this theory was not developed for systems where the cross-linking molecule occupies a large weight fraction in the sample. The theory is only strictly applicable to systems for zero-length or short cross-linking molecules. In the current situation, PEG is a flexible molecule and typically forms weak elastic gels, and the elastic deformation observed during compression of gels with high PEG content is likely caused by the less rigid PEG molecule being deformed rather than the stiff alginate.[34]

There is currently no theory that adequately addresses hydrogels cross-linked with other large molecules. Network properties such as cross-linking density and the molecular weight between cross-links (M_c) can be analyzed directly from measurements of the elastic modulus or the equilibrium swelling ratio under conditions where the rubber-elasticity theory holds.[28] However, these models include assumptions that are unrealistic in many systems. Thus, the values predicted from the theory used in current study are only indicative, and a more sophisticated model needs to be developed to describe these types of networks. Nonetheless, the order of magnitude of the experimentally determined M_c's agreed reasonably well with the theoretical values up to a cross-linking density that coincides with the highest modulus obtained for each cross-linking molecule. These results suggest that the model based on the rubber-elasticity theory is generally valid as long as the theoretical M_c is higher than the molecular weight of the cross-linking molecule, but not above this point. A number of theoretical models have been proposed that consider additional contributions (e.g., network imperfections) to describe more realistic networks,[35,36] but none consider the cross-linking by a second macromolecule. The utilization of more complex models proposed by Peppas and Merrill[37] for the systems used in this work confirmed the same trend in M_c (data not shown).

Conclusion

Alginate hydrogels were prepared by covalent cross-linking reactions with PEG−diamines of different molecular weights. We were able to control the mechanical properties of the alginate hydrogels not only by varying the chain length of the cross-linking molecule but also by changing the weight fraction of the cross-linking molecule in the hydrogel. The longer cross-linking molecules are apparently more efficient in the formation of intermolecular cross-links as opposed to intramolecular cross-links. However, as the length of the cross-linking molecule approaches the molecular weight between cross-links (M_c), the properties of the hydrogels become significantly influenced by the nature of the cross-linking molecule. This effect of the cross-linking molecules on the hydrogel properties also suggests other means to control specific properties of two-component hydrogels (i.e., hydrophilic/hydrophobic balance). The finding of this work may impact covalently cross-linked hydrogels for biomedical applications as well as other research areas where polymer networks are of importance and one wants to alter the mechanical properties by introduction of a second macromolecule.

Acknowledgment. This work was supported by Reprogenesis, Inc., and the NIH (R01 DE13033). We also thank Prof. F. Filisko for his helpful discussions.

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MA990514M