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PAPER

Real-time *in situ* rheology of alginate hydrogel photocrosslinking†

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The reaction dynamics of biodegradable, photocrosslinkable sodium alginate hydrogels are studied by *in situ*, dynamic rheology. Alginate, chemically-modified with methacrylate groups, crosslinks by ultraviolet (UV) light exposure in the presence of a photoinitiator. The gel formation is monitored during UV irradiation from a light emitting diode (LED) bottom plate fixture on the rheometer. Material properties of the hydrogels, including gel points and relaxation exponent, are evaluated using the Winter-Chambon criteria. We also report a new, complementary empirical method for determining the gel point from the reduction in sample strain at the onset of gelation, *via* monitoring the strain curve. In addition, the crosslinking dynamics and hydrogel moduli are altered by changing the UV irradiation intensities (3–15 mW cm^{-2}) and degree of methacrylation (5–25%). Dynamic rheological measurements of hydrogels as described in this paper are a potentially powerful tool to elucidate the dynamics of gelation and predict mechanical properties. This technique may aid in the design of polymer formulations with light-reactive chemical species, which have tunable properties that can be matched to a range of applications, including regenerative medicine.

Introduction

In the rapidly evolving field of biomedical engineering, hydrogels have exciting possibilities as functional biomaterials. These insoluble, three dimensional (3D) networks of crosslinked polymers can be swelled by aqueous solutions and can have customizable shapes, mechanical stability, and rates of degradation, making them promising materials for drug delivery and regenerative medicine.[1] Drugs and cells can be incorporated within hydrogels and released or delivered *in vivo* to specific targeted areas.[2] Of particular interest for *in vivo* applications are hydrogels made from naturally occurring polymers, due to their low toxicity and biodegradability.[3]

Hydrogels of sodium alginate, a polysaccharide derived from brown algae, have been used extensively as tissue engineering scaffolds[4] and drug and cell delivery[1,5] vehicles. Alginate hydrogels can be formed by crosslinking the biopolymer ionically with divalent cations (*e.g.*, Ca^{2+})[6] or chemically (*e.g.*, glutaraldehyde).[7] While alginate hydrogels made by these two methods have shown promise, both crosslinking approaches also have drawbacks. Ionically-crosslinked alginate hydrogels are limited by their range of mechanical stability and uncontrolled rate of degradation.[8] On the other hand, reagents used to chemically crosslink alginate hydrogels may have cytotoxicity concerns.[9]

An alternative chemical crosslinking approach entails the use of ultraviolet (UV) radiation to initiate free radical polymerization. These photocrosslinkable materials have been shown to be versatile and promising for use as tissue engineering matrices and controlled delivery platforms for a variety of applications (*e.g.*,[4,10–13]). Hydrogels that are photocrosslinkable can be injected *in vivo* with cells[14] and/or bioactive factors[15,16] to a target region (*e.g.*, damaged tissue) and then crosslinked with brief exposure to UV light in the presence of a photoinitiator. Furthermore, by varying the number of reactive groups, the degree of crosslinking of the hydrogels can be tailored to yield specific mechanical properties and degradation rates that resemble natural tissues and match desired cell proliferation rates, respectively.[2] Since tissues have a broad range of moduli (~1 kPa, liver to ~40 GPa, bone),[11] a material that can achieve a wide range of stiffness without significantly changing its chemistry and structure will be useful to study effects of scaffold modulus on cell behavior. Recently, UV light crosslinkable alginate hydrogels with controllable mechanical and degradation properties have been synthesized.[17] Alginate was made photocrosslinkable by coupling methacrylate groups to carboxylic acid moieties on the material backbone using carbodiimide chemistry. Hydrogels from methacrylated alginate (MAALG) are degradable *via* hydrolysis and encapsulated cells show good viability.[17]

While previous work has evaluated the mechanical properties of crosslinked MAALG hydrogels formed from alginate with

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a range of methacrylation, a systematic characterization of these properties during hydrogel formation has not been performed. This analysis is important because of the interplay between kinetics of crosslinking reactions and mechanical properties of the developing hydrogel. For example, the sample irradiation can be terminated when the hydrogel first approaches its plateau modulus to avoid irradiating beyond the completion of the crosslinking reaction, thus reducing the total dosage of exposure. In this study, the gelation of alginate involves of a series of chemical reactions as depicted schematically in Fig. 1. The reaction begins with the formation of free radicals by a photoinitiator that has been irradiated with UV light, which in turn, generate free radical intermediates on the methacrylated alginate. The initiated alginate chains can react with other unsaturated carbon-carbon bonds in the vinyl methylene groups, to form a series of crosslinks. The reaction is stopped by coupling or disproportionation of the radicals.[17,18]

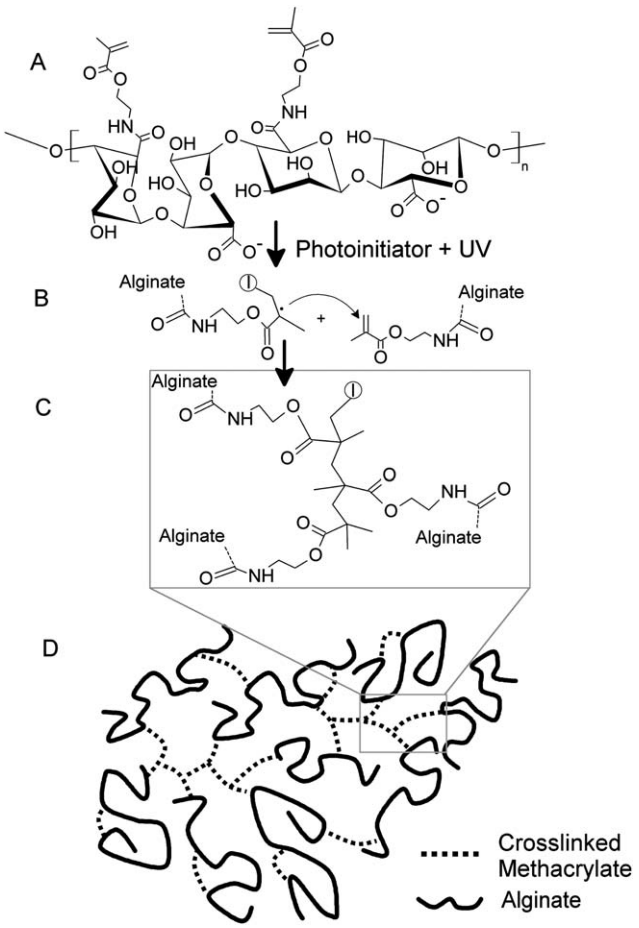

Fig. 1 Schematic illustration for the reaction scheme of methacrylated alginate hydrogels. After UV exposure, a photoinitiator can react with the tertiary carbon in the vinyl methylene group to form a radical, I, which initiates the polymerization (A). The radical group propagates through unreacted carbon-carbon double bonds (B) to form a network of crosslinked polymer chains (C). The reaction is stopped by coupling of the radicals. Crosslinks range in type and size, from heterogeneous lengths of methacrylate groups connecting alginate chains, to intramolecular bonds within the same alginate chain (D).

Dynamic rheology is an established technique for monitoring the kinetics of a crosslinking reaction. Changes in the rheological properties of the material reflect the formation and density of crosslinks.[19] Material properties, such as the elastic (G') and viscous (G'') moduli, can be detected using small amplitude oscillatory shear, with minimal disruptions to the chemical reaction. In addition, the evolution of a photocrosslinking reaction can be monitored *in situ* using a specialized UV source fixture.[20–25] Recently, UV rheometer setups were used to examine photocurable, biodegradable hydrogels with uses in regenerative medicine and biotherapeutics.[26–30] *In situ* rheological techniques are advantageous for these types of functional biomaterials, in order to help tailor the crosslinking kinetics and mechanical properties to the desired applications. A photopolymerizable material can be characterized in a rheometer-UV light source setup while the sample is irradiated, which captures the transition from sol to gel. The dynamic moduli of the curing material can accurately predict the gel point, signifying the time at which networks span between the plates of the rheometer.[19] The precise time when the onset of gelation occurs can be predicted using the Winter-Chambon criteria, which is applied to the loss tangent, tan δ.[31]

$$\tan\delta = G''/G' = \tan(n\pi/2) \quad (1)$$

n is the relaxation exponent and ω is the oscillation frequency. At the gel point, the loss tangent is independent of frequency. Thus, curves of tan δ for a material undergoing crosslinking measured at different frequencies will intersect at one point, corresponding to the gel point. For a polymer gel with relaxation exponent of 0.5, the gel point will correspond by the G'/G'' crossover on a time sweep.[32]

In this study we use dynamic rheology to study the crosslinking reaction of UV light crosslinkable, methacrylated alginate. MAALG macromers were reconstituted in cell culture media containing a water-soluble photoinitiator, allowing for incorporation of cells and ready use as tissue scaffolds. The evolution of rheological material properties were monitored *in situ* using a UV photocuring bottom plate fixture with a light emitting diode (LED) radiation source. With this setup, the sample was irradiated uniformly while experiencing a constant oscillatory small-amplitude stress. Rheological material properties, including G', G'', tan δ, and the complex viscosity, η*, of MAALG were characterized during gelation. Gel points were determined from frequency sweeps at discrete times during the reaction, as well as using the intersection of tan δ curves at different oscillatory frequencies. The relaxation exponent, n, was estimated from the slope of the elastic modulus *versus* frequency curve near the gel point. In addition to the Winter-Chambon criteria, we report an alternative method for determining the gel point using sample strain. Making use of stress-controlled experiments, we can monitor the rapid reduction in sample strain that occurs near the gel point. While this method is not as precise as Winter-Chambon approach, it is useful for crosslinking systems with fast gelation kinetics with rapid increases in G' and poor G'' signals beyond the crossover. In addition, we study the effects of the degree of methacrylation on alginate gel points and moduli. Alginates with 5–25% methacrylation, corresponding to the percent of alginate monomer subunits with methylacrylate groups, were evaluated.

The selection of the crosslinking conditions in this study were based on the intended uses of MAALG hydrogels to encapsulate cells and temperature-sensitive materials such as bioactive factors. In particular, the gelation kinetics was varied using relatively low UV intensities (3 to 15 mW cm^{-2}). UV intensities much greater than 15 mW cm^{-2} may lead to detrimental temperatures for cells due to the exothermic effects of the polymerization.[33] UV irradiation may also damage the DNA in cells.[34] In addition, free radicals from the photoinitiation can be detrimental to cell membranes and proteins.[35,36] In this study, we selected a commercial photoinitiator that has been previously evaluated in cell photoencapsulation studies.[36,37] Moreover, the crosslinking conditions, materials, and concentrations in this study have been closely matched to our previous work,[17] in which these controlled variables did not affect cell viability for seven days in methacrylated alginate hydrogels.

The characterization of MAALG hydrogels by UV rheological techniques has potential implications beyond this investigation. Changing the degree of methacrylation, and UV intensity and exposure time are methods to manipulate the rate of gelation and gel moduli, and tailor the hydrogel mechanical stability and rate of degradation. Thus, these are important parameters to consider when forming hydrogel tissue scaffolds for biomedical applications.

Experimental details

Materials

Irgacure D2959 photoinitiator and Dulbecco's Modified Eagle Medium with low glucose (DMEM) were used as received from Sigma Aldrich (St. Louis, MO). Photocrosslinkable alginate (5–25% (actual) methacrylation) was prepared by a method described previously.[17] In brief, 5Mrad irradiated Protanal LF 20/40 alginate (52 kDa, FMC Biopolymer, Philadelphia, PA) was dissolved in an MES buffer solution (Sigma Aldrich, St. Louis, MO). N-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (NHS and EDC, Sigma) were added to activate the carboxylic acid groups of the alginate, after which 2-aminoethyl methacrylate (AEMA, Sigma) was added and allowed to react for 24 h. The molar ratio of NHS: EDC:AEMA was maintained at 1 : 2 : 1, varying the amount of AEMA to achieve varying degrees of methacrylation. The product was precipitated in excess acetone, dried under reduced pressure, rehydrated in ultrapure deionized water (diH_2O), and dialyzed against diH_2O for three days. It was then treated with activated charcoal, filtered, and lyophilized until dry.

Preparation of solutions

Solutions of the photocrosslinkable alginates were prepared by dissolving the photoinitiator Irgacure D2959 (0.05% w/v) and methacrylated alginate (2% w/v) in DMEM. Solutions were kept in the dark to prevent sample degradation and/or reaction prior to measurements.

Rheological measurements

Rheological experiments were performed with a TA Instruments (New Castle, DE) G2 stress-controlled rheometer with UV photocuring bottom plate fixture with temperature controlled bath (Supplemental Figure 1†). Light emitting diodes (LEDs) within the fixture emitted UV radiation at 365 nm wavelength through a clear acrylic, UV transparent window. Radiation intensity was fixed throughout each experiment and values of 3–15 mW cm^{-2} were investigated. The fixture was calibrated using a Silver Line UV radiometer at the top of the quartz window, the same position as the sample. The instrument manufacturer has measured the irradiance uniformity to be within 5–7% of the specified value, across the surface of the plate.[38] A 20 mm aluminum parallel plate was used for the top geometry. Three different gap sizes (0.5, 1, 1.5 mm) were evaluated to test for possible wall slip (Supplemental Figure 2†). For the rest of the study, the gap was maintained at 0.5 mm. All measurements were made at 22 °C, 3–24 h after preparing solutions. Solution gelling behavior was monitored using dynamic oscillatory time and frequencies sweep experiments. Samples were exposed to UV radiation at the start ($t = 0$) of each time sweep. Throughout the time sweep, constant oscillations were applied at a fixed frequency (1, 5, or 10 rad s^{-1}) with a stress of 0.05 Pa, which was in the linear viscoelastic (LVE) regime of the material. UV exposure was terminated at the completion of each time sweep, after which frequency sweep experiments were performed. Stress sweeps (data not shown) were also performed at the conclusion of the dynamic studies to verify that the stress was in the LVE regime. Rheological measurements were duplicated to ensure repeatability within ±5% error.

Results and discussions

Evolution of crosslinking reaction

Steady state and dynamic rheology was employed to study alginate gelation kinetics. Prior to UV exposure, the solutions exhibited Newtonian behavior, with a shear viscosity on the order of magnitude of the solvent, ~0.007 Pa s (Supplemental Figure 3†). However, the solution viscosity increased by several orders of magnitude after UV photocrosslinking. Because steady shear rheology can break down the structure of a sample, small amplitude oscillatory experiments were used to monitor the crosslinking reaction *in situ*. The evolution of the elastic (G') and viscous (G'') moduli for alginate with 11% methacrylation are shown in Fig. 2. The moduli were measured as a function of frequency after exposure with 5 mW cm^{-2} UV radiation. Due to the low viscosity, the G' and G'' signals are scattered for $t < 500$, which may be due to inertial effects from the top geometry. G'' was an order of magnitude greater than G' at low frequencies after exposure for 900 s (15 min) and both moduli were frequency dependent, indicating a pregel. After 1200 s (20 min) the sample was slightly past the gel point, with G' and G'' near parallel and on the same order of magnitude. The value of n, the relaxation exponent for the gel, is ~0.5, which was approximated from a power law relationship ($G', G'' \sim \omega^n$) on the data beyond the gel point. After 1800 s (30 min) the sample was highly crosslinked, as G' became independent of frequency and more than two orders of magnitude greater than G''.

The transition from sol-to-gel can also be shown from plots of η^* *versus* frequency. The appearance of the η^* curves changed as the crosslinking reaction evolved. (Fig. 3) After 900 s, the slope

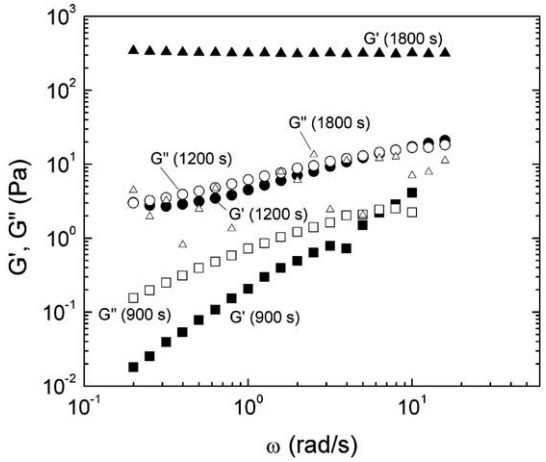

Fig. 2 Elastic (G′) and viscous (G″) moduli of photocrosslinkable alginate (11% methacrylation) as a function of frequency plotted after different exposure times with 5 mW cm^{-2} UV radiation. At 900 s the solution is a pregel, with G″ is greater than G′ at low frequencies and both moduli are frequency dependent. At 1200 *s* G′ and G″ are parallel and have similar values across most frequencies, indicating the material is a postgel. After 1800 s G′ is frequency independent and more than two orders of magnitude greater than G″, indicating a highly crosslinked sample.

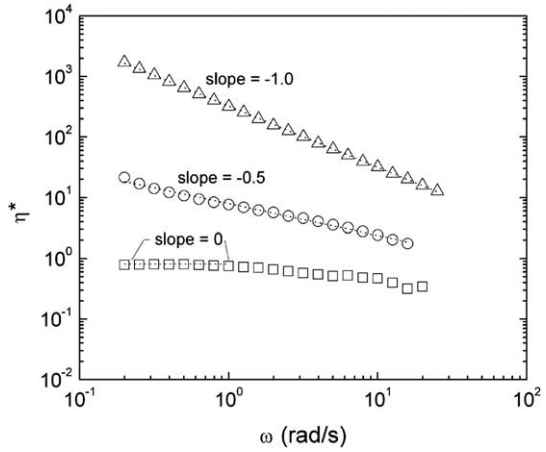

Fig. 3 Dynamic viscosity of photocrosslinkable alginate as a function of frequency plotted after 900 (□), 1200 (○), and 1800 (△) s of UV exposure with 5 mW cm^{-2} UV radiation. Slopes at low frequencies decrease from 0 to −1, indicating the evolution of sol to gel.

was zero at low frequencies and then became shear thinning. In comparison, the η* curves were shear thinning for all frequencies after 1200 and 1800 s exposure time, with slopes of −0.5 and −1.0, respectively. A decrease in dynamic viscosity can indicate the material is a postgel or solid, which confirms the onset of gelation as predicted by G′ and G″ in Fig. 2.[39] In addition, the relaxation exponent of the gel can be estimated from the slope of the complex viscosity at the gel point, $\eta^* \sim \omega^{n-1}$. The η* curve after 1200 s of exposure and within 100 s of the G′/G″ crossover has a slope of −0.5, and relaxation exponent ∼0.5, which is consistent with the value obtained from the frequency plots near the gel point in Fig. 2. The relaxation exponent is indicative of the gelation of large, linear flexible molecules.[19]

Another representation of the crosslinking reaction is shown as a function of time with a constant oscillation frequency (1 rad s^{-1}). (Fig. 4) The sample was irradiated over the duration of the experiment ($t \geq 0$). G″ exceeded G′ during the early stages of the free radical polymerization as the sample was in a pregel state. However, G′ surpassed G″ after 1100 s (18.3 min), near the gel point, and the elastic behavior of the sample was dominant for the subsequent duration of the experiment. Near this time point, a network of crosslinks formed between the two parallel plates on the rheometer. The G″ signal falls off after the gel point, indicating the sample behaved like a highly elastic solid and viscous liquid character of the sample was difficult to detect. As the crosslinking reaction progressed, the reaction rate decreased, as indicated by the decrease in slope of the G′ curve (∼1300 s). The decrease in rate was attributed to the reduction in the number of accessible or mobile unreacted species as the reaction approaches completion.[40] The elastic modulus increased by four orders of magnitude over the course of the reaction, revealing the complex nature of the material. The formation of methacrylate bonds in crosslinked alginate hydrogels resulting from UV irradiation was verified by ^{1}H NMR and reported previously.[17]

Gel point

The sample gel point was examined using the Winter-Chambon criteria by evaluating time sweeps at different frequencies. Fig. 5 shows time sweeps for alginate with 11% methacrylation (MAALG-11) with 5 mW cm^{-2} radiation intensity; tan δ curves for frequencies 0.5, 1, 5, and 10 rad s^{-1} intersect between 1080 and 1140 s, corresponding to the gel point region. The loss tangent values are 1.2–0.6, which correspond to relaxation exponents 0.3–0.6 with 95% confidence, calculated by eqn (1). Taken together the collective efforts for obtaining n at the gel point, including dynamic moduli, complex viscosity, and tan δ methods, we estimate that the n value is ∼0.5, which is

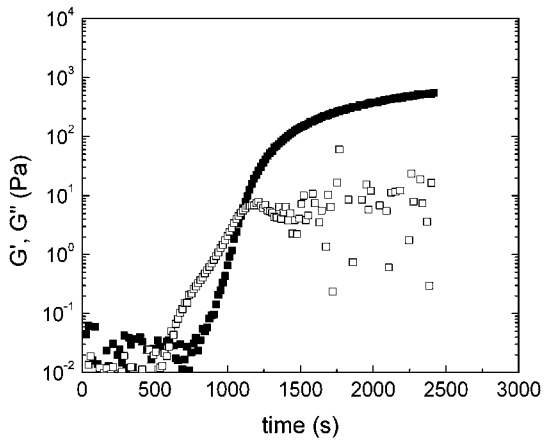

Fig. 4 Time dependent rheology of alginate with 11% methacrylation. Beginning at t = 0, sample was exposed to UV radiation with 5 mW cm^{-2} intensity and 1 rad s^{-1} oscillation with 0.05 Pa stress. The elastic (G′,■) and viscous (G″,□) moduli are plotted as a function of time. The gel point time (t_c) is 1080.8 ± 32.2 s, estimated by the G′/G″ crossover, and the corresponding modulus is 11.2 ± 4.0 Pa. (Means ± standard deviations are reported, sample size = 6.)

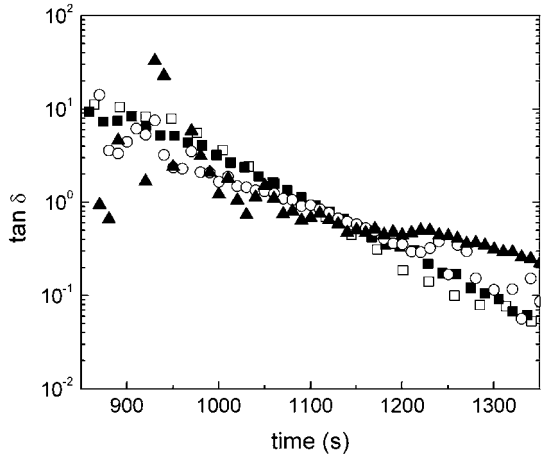

Fig. 5 Evolution of tan δ for 11% methacrylated alginate solution with 5 mW cm^{-2} UV irradiation using 0.5 (□), 1 (■), 5 (○), and 10 rad s^{-1} (▲) frequencies. The four curves intersect between 1080 and 1140 s, corresponding to tan δ values 1.2–0.6.

indicative of linear, flexible polymers,[19] such as the methylacrylated alginate from our study. The gel point for polymer gels with n ~0.5 can be conveniently approximated from G′/G″ crossover in time sweeps. While the crossover point may not correspond precisely to the gel point, we expect the two values to be of similar magnitude.

We identified another empirical method to characterize the onset of gelation by simply monitoring the sample strain during the photocrosslinking reaction, exploiting the fixed stress experimental setup. This new approach is advantageous for reaction systems, such as ours, which have a narrow window to examine the tan δ curves due to a rapid change in modulus and poor G″ signal beyond the gel point. Curves of % strain *versus* time are plotted at the same four frequencies from the previous section (0.5 1, 5, 10 rad s^{-1}). (Fig. 6) Near the onset of gelation, the sample strain rapidly decreases due to an increase in modulus. Interestingly, the curves for all four frequencies tend to overlay in this decreasing strain regime. While this region of steep strain decline provides a window for the gel point, the inflection point of the composite strain curve seems to correspond to the gel point (~1100 s) and provides a convenient way to pinpoint it. (Fig. 6b) A spike in zero shear viscosity occurs at the gel point,[19] which we speculate is related to a minimum slope for the strain. The inflection point approach seems to work well to predict the gel point at lower frequencies (0.5 and 1 rad s^{-1}) but not so at higher frequencies. At 5 and 10 rad s^{-1}, the strain curves display inflection points beyond the gel point. Nevertheless, we find that the method accurately predicts gel points for the majority of the samples and conditions investigated that were evaluated at 1 rad s^{-1} (see next sections.) Although not as accurate and versatile as the tan δ method to predict gel point, we believe that this is an interesting, complementary approach that can be applied to indicate the crosslinking reaction dynamics using a stress-controlled rheometer. Please note that at 5 and 10 rad s^{-1}, the strain curve reaches a maximum before decreasing. Further studies to investigate the peak strain appearance with other photocrosslinkable polymers are ongoing.

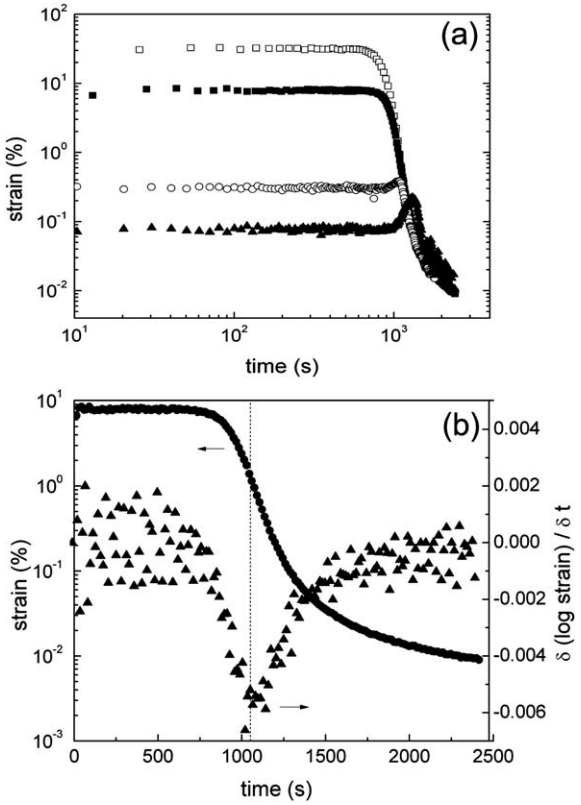

Fig. 6 (a) Strain on MAALG-11 throughout the crosslinking reaction, shown at 0.5 (□), 1 (■), 5 (○), and 10 rad s^{-1} (▲) and a 0.05 Pa constant stress. Strain reduces at onset of gelation. (b) Strain (●) and derivative of log strain (▲) at 1 rad s^{-1} plotted as functions of time. The dashed line marks the inflection point of the strain curve, the minimum of the derivative, and corresponds to the gel point region.

Effect of UV intensity

The kinetics of gelation and the gel mechanical properties for photocrosslinkable alginate can be manipulated with UV intensity. In turn, the gel properties influence cell proliferation, migration, and differentiation on gels used as tissue scaffolds.[14,41,42] Furthermore, past studies (*e.g.*, ref. 14) with UV curable systems commonly report time to gelation at one UV dosage. However, variability in UV sources between laboratories may cause repeatability concerns over the extent of the crosslinking. In our study, the rheometer's bottom plate with built-in UV light source is capable of easily altering the irradiation level and is ideal for evaluating the effects of changing UV intensity. Fig. 7a shows time sweeps for MAALG-25 as a function of UV intensity. The gel point varies from 354 to 1471 s by reducing radiation levels from 15 to 3 mW cm^{-2}, which correspond to total radiation dosages ranging from 5310 to 4413 mJ cm^{-2}, respectively. The relationship between UV intensity and crossover time is plotted as an inset in Fig. 7b. A line fit to the experimental data shows a power law dependence, $t_c \sim I^{-0.85}$, which is in agreement with a relationship previously reported with a different crosslinking reaction (*i.e.*, urethane-based thiols).[20] Decreasing UV intensity reduces the number of free radicals formed by the photoinitiator, which also reduces rate of photocrosslinking. The effect of UV intensity on gelation is also apparent when

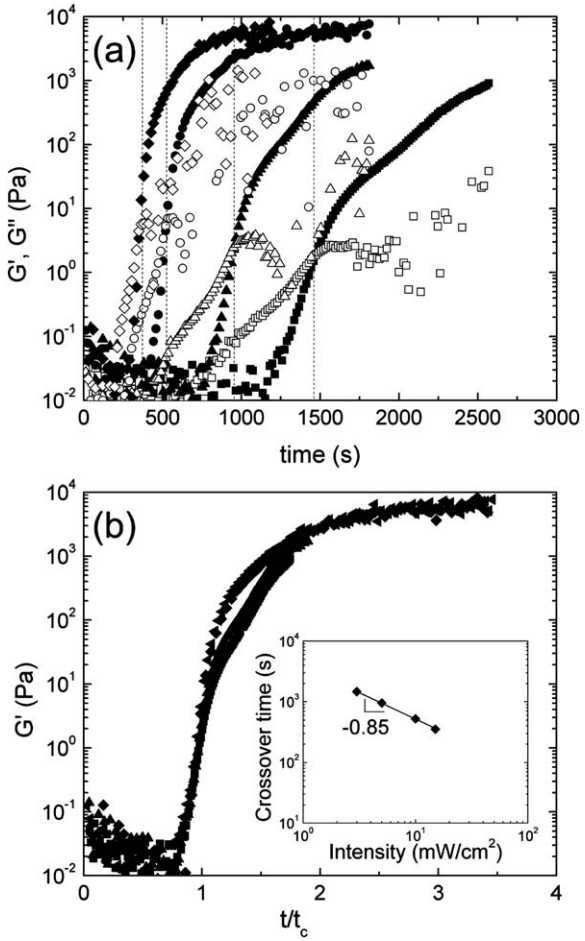

Fig. 7 (a) Evolution of elastic and viscous moduli of alginate with 25% methacrylation at 3 (■□), 5(▲Δ), 10(● ○), and 15 (◆ ◇)mW cm^{-2} doses of UV irradiation. Dashed lines indicate G′, G″ crossover and gel points. (b) The elastic moduli collapse when the dependent variable is scaled by the crossover time, as t/t_c. Inset graph shows crossover times plotted *versus* UV intensity. The line is a power law curve fit.

comparing the shape of the elastic moduli curves. In samples with 10 and 15 mW cm^{-2} exposure, G′ increases by two orders of magnitude within ~200 s after the gel point. The rapid change suggests that there is a surplus of reactive species dispersed throughout the sample at the onset of gelation that continue to react as the material transitions to a highly crosslinked gel. In comparison, the elastic moduli of the samples with 3 and 5 mW cm^{-2} of exposure increased at a slower rate, and did not approach the same modulus (~5000 Pa) after 1800 s of monitoring the samples. When scaled with the crossover time, the curves collapse to one master curve at the early stages of gelation. (Fig. 7b) However, beyond the gel point ($t/t_c \geq 1$), the data deviate from one master curve. This suggests that while the hydrogel formation mechanism is intensity independent up until the gel point, it is not so following it. While UV intensities less than 5 mW cm^{-2} were capable of initiating the crosslinking reaction in MAALG-25, the resulting network structures were apparently weaker than compared to the higher UV intensities. One explanation may be the result of differing crosslinking mechanisms. The hydrogels that formed at the lower intensities may have a greater concentration of intramolecular crosslinks,[43] thus reducing the final modulus as compared to the higher UV intensities. The ability to manipulate the network structure of the hydrogels is important for biomedical applications, such as drug delivery, where the release rate is influenced by the density of the crosslinks.[2]

As we discussed in the previous section, the inflection point (δ (*log strain*)/$\delta t = 0$) or the minimum slope in the strain plotted *versus* time can be used to identify the gel point. Shown in Fig. 8, strain decreases at the onset of gelation for each UV intensity. The highest UV intensities (10, 15 mW cm^{-2}) yielded the greatest reduction in strain. As expected, highly-crosslinked hydrogels will have smaller strains than weaker networks that were inducted by low UV intensities (<10 mW cm^{-2}). As observed previously for a sample evaluated at 1 rad s^{-1}, the inflection points of the each strain curve matches t_c. Thus, the inflection point method is valid for alginate hydrogels with a range of crosslinking dynamics.

Varying degree of methacrylation

In addition to UV intensity, the degree of methacrylation is essential to material properties of photocrosslinkable alginate hydrogels, affecting their performance as tissue scaffolds. We have previously shown that degree of methacrylation correlates with the swelling and rate of degradation of the hydrogel.[17] In regenerative medicine, scaffold modulus and rate of degradation affect the formation of new tissues.[4] In our study, we characterized the evolution of gelation for photocrosslinkable alginate with 5, 7, 11, 19, and 25% methacrylation. Compared to hydrogels made with varying UV intensity, hydrogels with differing degrees of methacrylation do not approach the same terminal moduli. (Fig. 9a) As expected, reducing the crosslink density of the hydrogel results in a lower modulus. Furthermore, the total radiation dosage at the crossover time ranges from 7750 to 5240 mJ cm^{-2} with increasing degree of methacrylation. These dosage values are greater than or equal to the values reported in the intensity study. Based on the collective data sets of the intensity and methacrylation conditions evaluated, the

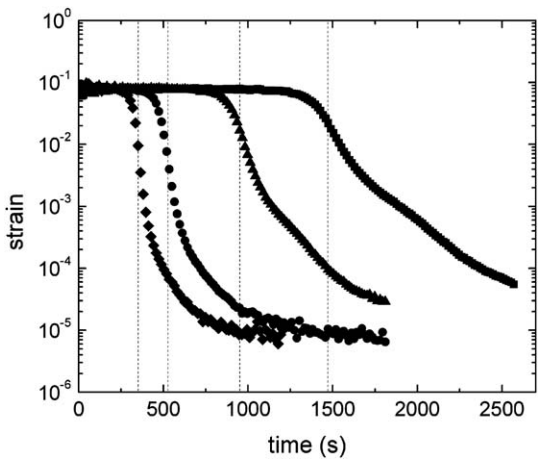

Fig. 8 Sample strain as a function of time for 3(■), 5(▲), 10(●), and 15 (◆) mW cm^{-2} intensities. Dashed lines indicate gel points, which match with the inflection points of the experimental data.

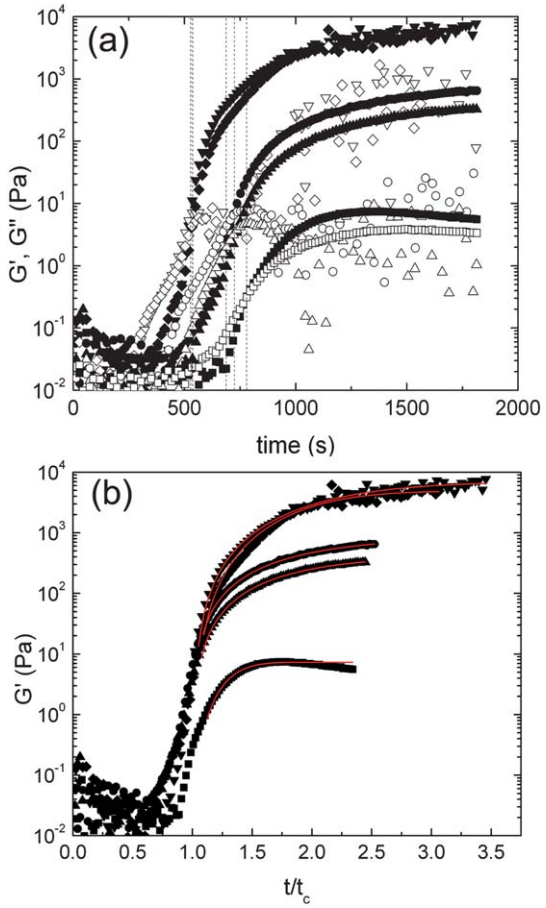

Fig. 9 (a) Elastic and viscous moduli as a function of time for photo-crosslinkable alginate with 5(■ □), 7(▲ Δ), 11(● ○), 19(◆ ◇), and 25% (▼,▼) degrees of methacrylation. UV irradiation with 10 mW cm^{-2} intensity was applied for $t \geq 0$. Dashed lines mark crossover times, which are approximately the gel points. (b) Time sweeps scaled with crossover times and fitted. Solid lines are curve fits from $G' = G'_\infty(1 - \exp[-\alpha(t/t_c - 1)^\beta])$. For 5, 7, 11, 19, and 25% degrees of methacrylation, G'_∞ = 7.3, 410, 846, 4859, 6860 Pa; α = 8.1, 1.0, 0.8, 0.8, 0.6; β = 2.0, 1.5, 1.4, 2.2, 1.9, respectively.

irradiation dosage required to form MAALG hydrogels is > 4413 mJ cm^{-2}.

Since the elastic moduli did not reach to the plateau moduli in the time frames evaluated in this study, curve fits were applied to estimate the terminal moduli. The time sweeps were scaled by crossover times and fit using an empirical model developed by Cao *et al.*:[44]

$$G' = G'_\infty(1 - \exp[-\alpha(t/t_c - 1)^\beta]) \quad (2)$$

G'_∞ is the plateau modulus, and α and β are fitting parameters. Shown in Fig. 9b, model curves match well with the experimental data for time beyond the gel point ($t > t_c$). Results of G'_∞ obtained from this plot together with t_c are plotted as a function of degree of methacrylation in Fig. 10. Several features are apparent from Fig. 9 and 10. First, the plateau moduli estimates vary by over three orders of magnitude with 5–25% degree of methacrylation. If used as scaffolds, the moduli of the alginate hydrogels could match the stiffness of a wide range of tissue

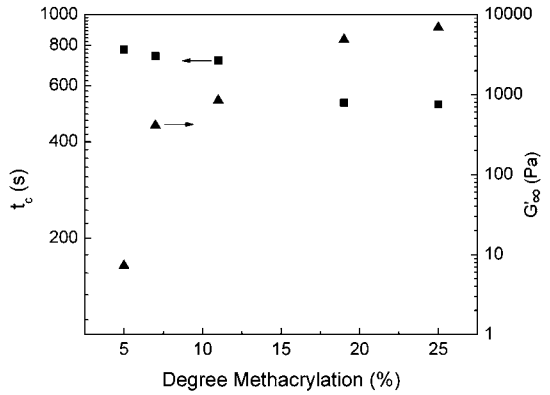

Fig. 10 Crossover times and plateau moduli for alginate hydrogels with different degrees of methacrylation. Plateau modulus was estimated from curve fits described in Fig. 9.

types, including liver, breast, or arterial wall tissues.[11] Second, while we see in Fig. 10 that the moduli variation is significant (over three orders of magnitude) with degree of methacrylation, the gel point is not as sensitive, varying only by ~50%.

Finally, when comparing individual samples, the effects of methyacrylation on rheological behavior vary. The terminal moduli of the samples with the highest degree of methacrylation, MAALG-19 and 25, are at least one order larger than the other MAALG-7, 11 samples, and significantly more than the MAALG-5 samples. Increasing the concentration of available reactive groups corresponds to a higher reaction rate and crosslink density in the termination of experiment. While the effects of degree of methacrylation on gel rheology were apparent when comparing MAALG-19 and 25 to other samples, the rheological behavior of these two samples has similarities. Specifically, they had similar gel points (530, 524 s) and comparable plateau moduli (6860, 4859 Pa), respectively. When comparing MAALG-5, 7, and 11, the gel points differ by up to 60 s between the samples, whereas the plateau moduli vary significantly. (Fig. 10) The predicted terminal moduli of MAALG-7 is half that of MAALG-11 (410, 846 Pa, respectively). Additionally, despite having the closest degrees of methacrylation of the samples investigated, MAALG-5 and 7 have stark differences. Specifically, the elastic and viscous moduli of MAALG-5(~7 Pa) are much smaller than MAALG-7, and remain on the same order of magnitude after the gel point. Furthermore, MAALG-5 shows a clear G″ signal >50 s beyond the gel point. As a result of the low methacrylate concentration, the MAALG-5 forms a weak gel, displaying some viscous tendencies beyond the gel point.[45] Moreover, a frequency sweep of crosslinked MAALG-5 reveals that the elastic and viscous moduli are on the same order of magnitude (Supplemental Figure 4†). A weakly crosslinked hydrogel can be advantageous in biomedical applications when more rapid degradation would be beneficial (*e.g.*, matching tissue growth rates[46]).

As in the previous section, the inflection point method was applied to the alginates with differing degrees of methacrylation. (Fig. 11) Strain decreases at the onset of gelation for MAALG-7, 11, 19, and 25, and the inflection points, or minimum slope values, matches t_c. The one exception is MAALG-5, which reaches maximum strain prior to decreasing, and is similar to strain

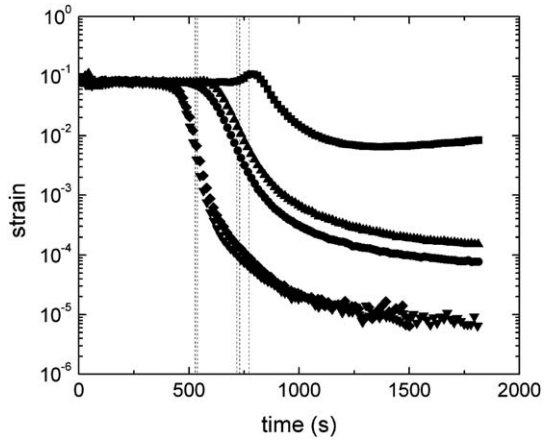

Fig. 11 Strain as a function of time for photocrosslinkable alginate with 5 (■), 7 (▲), 11 (●), 19 (◆), and 25% (▼) degree of methacrylation. Dashed lines indicate gel points, which match the inflection points for 7, 11, 19, and 25%.

curves at frequencies greater than 1 rad s^{-1} reported above. MAALG-5 formed a weak gel, which may have contributed to the deviation from the other samples.

Conclusion

In situ, small amplitude oscillatory shear rheology is an effective tool for monitoring the crosslinking reaction of photo-crosslinkable, methacrylated alginate hydrogels. The gel points were estimated using the Winter-Chambon criteria, which approximately matched the G'/G'' crossover. In addition, we identified an alternative approach to approximate gel points by monitoring the sample strain during crosslinking and observing its sharp decline near the gel point. Further refinement of the gel point was obtained from the minimum slope, or inflection point, of log strain *versus* time plot. We also evaluated the crosslinking reactions of alginates with differing UV irradiation exposure intensities and differing degrees of methacrylation. UV intensity varied with gelation time by a power law relationship, reminiscent of earlier work on acrylate systems. The elastic moduli of alginates with varying degrees methacrylation were fit with an empirical model, which estimated the terminal moduli. The terminal moduli were found to be quite sensitive to the degree of methacrylation varying by several orders of magnitude.

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