Controlling hydrogelation kinetics by peptide design for three-dimensional encapsulation and injectable delivery of cells

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A peptide-based hydrogelation strategy has been developed that allows homogenous encapsulation and subsequent delivery of C3H10t1/2 mesenchymal stem cells. Structure-based peptide design afforded MAX8, a 20-residue peptide that folds and self-assembles in response to DMEM resulting in mechanically rigid hydrogels. The folding and self-assembly kinetics of MAX8 have been tuned so that when hydrogelation is triggered in the presence of cells, the cells become homogeneously impregnated within the gel. A unique characteristic of these gel–cell constructs is that when an appropriate shear stress is applied, the hydrogel will shear-thin resulting in a low-viscosity gel. However, after the application of shear has stopped, the gel quickly resets and recovers its initial mechanical rigidity in a near quantitative fashion. This property allows gel-cell constructs to be delivered via syringe with precision to target sites. Homogenous cellular distribution and cell viability are unaffected by the shear thinning process and gel/cell constructs stay fixed at the point of introduction, suggesting that these gels may be useful for the delivery of cells to target biological sites in tissue regeneration efforts.

hydrogel | self-assembly | stem cell

Hydrogels are heavily hydrated materials finding use in tissue regeneration efforts as extracellular matrix substrates (1, 2). For example, preformed hydrogels inserted into cartilage (3–5), bone (6–9), and liver (10–12) defects in animal models show potential promise in aiding tissue repair in humans. In addition to preformed gels, “smart” polymeric systems are being developed that undergo solution–hydrogel phase transitions in vivo (13). In these systems, either aecal aqueous solutions of polymer or solutions containing desired cell type(s) are introduced at the tissue site. Subsequent gelation can occur by taking advantage of environmental differences between the polymeric solution and the in vivo environment such as temperature (14, 15), ionic strength (16, 17), or enzymatic activity (18). Alternatively, in vivo gelation can be accomplished by initiating the cross-linking of photopolymerizing polymer precursors by using cytocompatible photoinitiators (19, 20). These systems, which are introduced as liquids that subsequently gel after injection, offer the potential of minimally invasive material implantation by delivering solutions through a catheter into a small incision. Acellular systems result in gels designed to be infiltrated by cells from the surrounding tissue, whereas cellular systems afford gel/cell constructs that are designed to foster more immediate tissue regeneration. Both systems may contain growth factors and/or cytokines to enhance tissue regeneration (21).

Several material properties are commonly studied and often used to benchmark the potential success of a new material. Cytocompatibility of a material is normally studied by assessing material cytotoxicity, cell adhesion (attachment and subsequent morphological changes), proliferation, phenotype maintenance, and differentiation if progenitor cells are used. Material biocompatibility measures material-induced inflammation and immune response. Also, although not a necessary material attribute, biodegradability can be measured. Last, the desired bulk mechanical properties such as rigidity, elasticity, and compressibility, to name a few, are dependent on the specific biological application and are a direct consequence of the nano- and microstructure of the hydrogel. For many newly developed hydrogel materials, research mainly focuses on addressing the criteria outlined above.

However, for injectable “phase transition” materials that are designed to deliver cells to a wound site, additional and very important, practical criteria exist, which are challenging to meet. First, the gelation kinetics must be fast enough to ensure that cells become homogeneously incorporated within the matrix; it is becoming increasingly clear that cell density plays a role in modulating the behavior of delivered cells (4, 22). Gelating systems that afford an even distribution of encapsulated cells allow reproducible control over cell density within the matrix. In contrast, systems that gel slowly result in cell sedimentation and gross variation of the cell density throughout the matrix. Second, the spatial resolution with which a gel–cell construct can be introduced in vivo and its ability to remain localized at the point of introduction is of paramount importance. A possible limitation exists for material systems that are delivered to tissue defects as liquids; unless a well defined cavity exists that will contain the hydrogel precursor solution, leakage into/onto neighboring tissue is unavoidable and potentially harmful. For bone and cartilage repair, the implant site can be constrained to limit motion and periosteal flaps can be used to help spatially restrict material leakage (23). However, even for well defined osteochondral defects, multiple applications of the liquid precursor may be necessary (24). For other tissues, well defined cavities are not common. In sum, if widespread clinical use is anticipated, then an injectable gel–cell construct must be easily administered, must evenly distribute the delivered cells, and must stay localized at the site of introduction.

Results and Discussion

We have been developing a general hydrogelation strategy, based on the triggered self-assembly of peptides. The design of this system links the intramolecular folding of amphiphilic...
β-hairpin peptides to their propensity to self-assemble affording hydrogel material. Peptides are designed such that when dissolved in aqueous solutions, they exist in an ensemble of random coil conformations rendering them fully soluble. However, the addition of an exogenous stimulus results in peptide folding into a β-hairpin conformation that undergoes rapid self-assembly forming a β-sheet-rich, highly cross-linked hydrogel (25–31) (Fig. 1a). Peptides have been designed to fold and assemble in response to changes in pH (30) or ionic strength (25) or to the addition of heat (31) or light (27). Besides these stimuli, the addition of cell culture media to buffered solutions of unfolded peptide triggers folding, self-assembly, and ultimate gelation (28). Fig. 1a shows this process for the peptide MAX1, the parent peptide from which the structure-based design of MAX8 was derived to allow the three-dimensional (3D) homogenous encapsulation of cells and their subsequent delivery.

Because of electrostatic repulsion between positively charged lysine residues, these peptides remain unfolded in low ionic strength buffer at pH 7.4. However, folding can be triggered by screening some of the lysine-based charge with the addition of DMEM, which contains sufficient concentrations (~160 mM) of mono- and divalent inorganic salts to ensure effective screening. In the folded state, these peptides adopt a hairpin conformation composed of two β-strand sequences of alternating hydrophobic and hydrophilic residues (Lys and Val) flanking a tetrapeptide type II β-turn. These hairpins are amphiphilic molecules in which one face is hydrophobic and the other is hydrophilic. Folded hairpins self-assemble both laterally (via the formation of intermolecular H-bonds and van der Waals contacts) and facially (via the burial of the hydrophobic face of distinct hairpins) (Fig. 1a). Detailed structural characterization indicates that MAX1 gels are comprised of a network of fibrils rich in β-sheet (26, 29, 30). Each fibril is ~3 nm in width, consistent with the folded state of the molecule. Fibrils are physically cross-linked by noncovalent, hydrophobic interactions between the hydrophobic faces of assembled hairpins and local fibril entanglements. The fibril persistence length (distance between cross-link sites) ranges from ~10 to 200 nm. Cryo-transmission electron microscopy (cryo-TEM) and laser scanning confocal microscopy (LCSM) indicate that the gels are well hydrated on both the nano- and microlength scales and are microporous (30). Together, these material characteristics are attractive for tissue engineering/regeneration applications (1).

A unique feature of the hairpin gels is that when an appropriate shear stress is applied, the gel will shear-thin, resulting in a low-viscosity gel. However, after the application of shear has stopped, the gel quickly recovers its mechanical rigidity (Fig. 1a). Shear thin-recovery processes hold promise for minimally invasive material delivery (32–37). For example, alginate-based gels impregnated with fibroblast have been delivered s.c. into the backs of rats (32). However, the measured mechanical rigidity of these gel/cell constructs after syringe delivery indicates that the delivery process is severely detrimental to the mechanical integrity of the gel. In fact, this report concludes that cells may best be delivered as suspensions in alginate solutions that are gelled after syringe delivery.

Here, we report that hairpin hydrogelation can be triggered in the presence of C3H10t1/2 mesenchymal stem cells resulting in self-supporting, mechanically rigid gels that are impregnated with cells. This gel-forming process can be simply performed in a syringe. The resulting gel/cell constructs can then be shear-thin delivered to a targeted secondary site where they quickly recover to their original mechanical rigidity with location permanency. In this study, C3H10t1/2 cells are used as a model cell line because they are sensitive to their environment, thus providing a rigorous assessment of the delivery method with respect to cell viability.

When gelation is initially triggered, the rate of folding and self-assembly must be controlled to ensure homogenous cell incorporation. Gels that form too slowly or quickly would afford nonhomogeneous incorporation in which cells either sediment to the bottom or are trapped at the top of the syringe. Making multiple injections of a nonhomogenous gel/cell construct from a single syringe into different wound sites would result in grossly varied cell density at each injection site. After injection, gel recovery must be fast to ensure that after the viscous gel leaves the syringe and contacts the tissue, the gel quickly recovers and remains localized at the point of introduction. For example, Fig. 2a contains an LSCM cross-sectional view of C3H10t1/2 cells encapsulated within a 0.5 wt% MAX1 hydrogel. In this micrograph, cells contained within the interior of the gel are visualized from the side of the gel (along the y axis; depth of field is 920 μm); the top and bottom of the image shows the top and bottom of the gel, respectively. Cells are encapsulated by first dissolving 1.0 wt% MAX1 in buffer at pH 7.4. To this solution, an equal volume (50 μl) of DMEM containing 250,000 cells is added. The salt within the DMEM electrostatically screens the lysine side chain charges resulting in peptide folding and consequent self-assembly. This simple one-to-one addition of solutions produces gels impregnated with cells. However, it is clear in Fig. 2a that the hydrogelation kinetics of MAX1 are not fast enough to entrap the cells homogeneously because a majority of the cells sediment to the bottom of the gel sample well.

According to our model of folding and self-assembly, the eight positively charged lysine side chains of MAX1 must be accommodated on the hydrophilic face of the hairpin for the peptide to fold at pH 7.4. The kinetics of MAX1 hydrogelation should be hastened by making a point amino acid substitution on the hydrophilic face.
that lowers the overall charge density. By replacing the lysine side chain at position 15 with a negatively charged side chain of glutamic acid, the overall peptide charge state is lowered by 2. The resultant peptide, MAX8 (Fig. 1b) has a lower amount of positive charge to be screened and should fold and assemble much faster than MAX1 in response to identical cell culture conditions. Apart from this, the MAX8 hairpin could possibly be stabilized by cross-strand salt bridge interactions between the glutamic acid and cross-strand lysine residues in the self-assembled state. Importantly, Fig. 2 shows the LSCM image of a 0.5 wt% MAX8 hydrogel impregnated with C3H10t1/2 mesenchymal stem cells, clearly illustrating that the kinetics of hydrogelation are optimal for a nearly homogeneous distribution of cells; the depth of field (920 μm) is identical to that in Fig. 2a. The ability of MAX8 to homogeneously encapsulate cells is consistent and reproducible and appears to be independent of cell type; additional encapsulation experiments using a separate lot of C3H10t1/2 mesenchymal stem cells as well as hepatocytes (Hep G2) are provided in the supporting information (SI).

To further investigate the influence of the peptide’s charge state on folding and self-assembly kinetics, we used circular dichroism (CD) spectroscopy and oscillatory rheology. Fig. 3a shows the onset of mean residue ellipticity at 216 nm, indicative of β-sheet formation, as a function of time under solution conditions identical to those used for 3D cell encapsulation. The data clearly show that at 0.5 wt% MAX1 requires ~30 min to undergo complete β-sheet transformation, whereas MAX8 is complete in ~1 min. The difference in gelation kinetics is also observed by using oscillatory rheology (Fig. 3b) showing the onset of gel rigidity by monitoring the storage modulus (G’) versus time for 0.5 wt% MAX1 and MAX8 hydrogels. MAX8 forms a rigid hydrogel (G’ > 100 Pa) within the first 40 sec and further stiffens with time. A G’ of 100 Pa is sufficient to support cells within the 3D confines of the gel network. In contrast, MAX1 does not reach 40 Pa even after 60 min. The significant difference in storage modulus between the two peptide gels may be due to an increased number of cross-links and fibril entanglements formed as a result of MAX8 assembling at a faster rate.

Importantly, this increase in hydrogelation kinetics has little effect on the local nanostructure of the fibrils formed by MAX8 as compared with MAX1. This is important to assess because the nanostructure of assemblies formed from peptides are greatly influenced by sequence. For example, peptides of similar secondary structure but differing in amino acid composition and sequence have been shown to assemble into laminating fibrils (26, 38, 39), ribbons and tapes (40), and tubes (41–43), with each assembly morphology possibly having distinct affects on the bulk material properties. The β-hairpin peptides described herein self-assemble into a supermolecular structure composed of noncovalently cross-linked fibrils that are monodisperse in diameter and do not undergo lamination. TEM images highlighting the local morphology of the fibrils in each gel shows fibril diameters of ~3 nm for MAX1 (Fig. 4a) and MAX8 (Fig. 4b). These dimensions are consistent with the width of an individual hairpin in the self-assembled state. Together, the data suggest that MAX8 folds and self-assembles in a similar manner to that of MAX1, showing that rational modification of the net charge on the hydrophilic face can be used to control the kinetics of β-sheet formation and hydrogelation.

In order for gel/cell constructs to be delivered by syringe, MAX8 gels must exhibit shear-thinning behavior, yet recover quickly after delivery when they are no longer under shear stress. The ability of acellular MAX8 hydrogel to shear-thin and recover was assessed by oscillatory rheology. Cells were not included in these rheological experiments because the shear stress induced by the rheometer during shear-thinning (which is much greater than that encountered during syringe delivery) would result in cell lyses. In these experiments, shear-thinning at a 1,000% strain actually converts the gel into a liquid, whereas shear-thinning the gel via syringe only results in a low-viscosity gel. However, rheological data provide a quantitative assessment of shear-thin recovery kinetics. The data in Fig. 5a show that after shear-thinning in the rheometer, the hydrogel immediately recovers. In phase 1 of this experiment, hydrogelation is triggered by the addition of DMEM to a MAX8 buffered

Fig. 2. Encapsulation of mesenchymal C3H10t1/2 stem cells in 0.5 wt% MAX1 and MAX8 hydrogels. Shown are LSCM z-stack images (viewed along the y axis) showing the incorporation of cells into a MAX1 gel leading to cell sedimentation (a) and into a MAX8 gel resulting in cellular homogeneity (b). Cells are prelabeled with cell tracker green to aid visualization. (Scale bars: 100 μm.)

Fig. 3. CD spectroscopy and oscillatory rheology of MAX1 and MAX8 hydrogels. (a) Kinetics of β-sheet formation for MAX1 (squares) and MAX8 (triangles). The evolution of β-sheet is monitored during the solution-hydrogel phase transition by recording [θ]216 as a function of time for a 0.5 wt% peptide solution at 37°C after folding and self-assembly is initiated by the addition of DMEM (pH 7.4). Inset shows CD wavelength spectra characteristic of β-sheet structure for MAX1 and MAX8 hydrogels after the kinetics measurements. (b) DTS measurements of MAX1 (squares) and MAX8 (triangles) monitoring the evolution of storage modulus (G’) as a function of time for 0.5 wt% hydrogel at 37°C in DMEM (pH 7.4); frequency = 6 rad sec⁻¹, strain = 0.2%.

Fig. 4. TEM micrographs of MAX1 and MAX8 hydrogels showing the nanoscale of 0.5 wt% gel network prepared at 37°C with DMEM for MAX1 (a) and MAX8 (b) negatively stained with uranyl acetate. (Scale bars: 100 nm.) (Insets) Magnification of MAX1 and MAX8 fibrils that are ~3 nm in width. (Scale bars: 20 nm.)
solution, and the onset of \( G' \) is measured as a function of time. A \( G' \) of > 100 Pa is realized within 1 min with further stiffening of the network, showing a \( G' \) of 450 Pa at 5 min. In phase II, 1,000% strain is applied for 30 sec to the hydrogel resulting in the shear-thinning of the sample and the conversion of the gel to a liquid. In phase III, the applied strain is decreased to 0.2% and gel recovery is monitored. The kinetics of recovery are markedly faster than the initial gelation kinetics; recovering gels display a storage modulus of nearly 200 Pa within the first 5 sec of recovery and further stiffen with time. Data from six separate experiments indicate that the storage modulus of recovering gels is 90 ± 10% that of the initial modulus. The fast recovery kinetics are most likely due to the fact that many of the noncovalent cross-links of the gel network remain intact during shear-thinning and only a small fraction needs to reassemble for the gel to recover (Fig. 1a).

The recovery kinetics are little affected when cells are encapsulated in the network. Although recovery kinetics of MAX8 gel/cell constructs were not quantitatively measured by rheology, visually the gel rigidity immediately recovers when shear-thin delivered to a secondary container or surface via syringe. Fig. 5b Inset shows a 0.5 wt% MAX8 gel/cell construct that had been formed directly in a syringe. This construct was subsequently delivered to a LSCM well and imaged to assess the distribution of cells within the hydrogel network after shear-thinning. Fig. 5b shows that the cells still retain the homogeneous cell distribution established during initial gel assembly similar to that shown in Fig. 2b.

The effect of shear-thin delivery on cell viability was assessed by performing a Live-Dead assay on cells that had been encapsulated into a 0.5 wt% MAX8 hydrogel and subsequently syringe-delivered to an LSCM well (Fig. 5c). In this micrograph, cells contained within the interior of the gel are visualized by viewing from the top of the gel (along the \( z \) axis; depth of field is 90 \( \mu \)m). Three hours after the gel/cell construct had been syringe-delivered, calcien AM and ethidium homodimer were added to the top of the gel, which freely diffused through the gel network, staining live cells green and dead cells red, respectively. It is evident that the majority of the cells remain viable after delivery. In fact, the limited degree of cell death observed in Fig. 5c is nearly identical to that of the control experiment where cells were encapsulated in MAX8 hydrogel but not shear-thin delivered (see SI). The apparent variation in cell density between Fig. 5b and c is due to the fact that a 920- \( \mu \)m vs. a 90- \( \mu \)m depth of field is visualized in each figure, respectively. The smaller depth of field in Fig. 5c was used to allow sufficient resolution to detect dead cells.

Gel/cell constructs can be delivered with high precision that is limited only by the gauge of the syringe needle. Recovered gel/cell constructs are visually clear at these cell-loading densities, are self-supporting, and remain localized at the point of application even when agitated. The ability of the gel/cell construct to remain localized will undoubtedly depend on the surface (tissue) type. However, results of \textit{ex vivo} experiments are shown in Fig. 6. Fig. 6a shows MAX8 gels that have been syringe delivered to a horizontally oriented tissue culture-treated polystyrene surface and repositioned vertically for imaging, demonstrating gel location permanency. Fig. 6b shows a gel that had been delivered to a vertically positioned borosilicate surface; note that the gel does not run, demonstrating that they recover quickly after syringe delivery.

**Conclusion**

Results presented here demonstrate that peptide design can be used to generate hydrogel materials suitable for cell delivery. Peptide folding and self-assembly kinetics as well as shear-thin recovery kinetics have been tuned to allow for the homogenous encapsulation of C3H10t1/2 stem cells within the 3D network of a hydrogel. Resulting gel/cell constructs can be shear-thin delivered via syringe to target sites with little effect on the homogenous distribution of the cells. Cells remain viable during the encapsulation and shear-thin delivery process, and gel/cell...
constructs stay fixed at the point of introduction, suggesting that these gels may be useful for the delivery of cells to target biological sites in tissue-regeneration efforts. More broadly, this study suggests that peptide design can be used to control gelation kinetics and bulk material properties to address specific technologies that demand materials with customized properties.

Materials and Methods

Hydrogel Preparation. Peptides were synthesized through Fmoc-based solid phase peptide synthesis and purified to homogeneity as described in detail in the SI. To a vial containing 1 mg of peptide, 100 μl of 25 mM Hepes (pH 7.4) was added resulting in a soluble 1 wt% peptide solution. To this solution, an equal volume of DMEM supplemented with 25 mM Hepes (pH 7.4) was added to initiate self-assembly, resulting in a 0.5 wt% hydrogel. All hydrogels were prepared by this method unless otherwise stated.

CD. CD kinetic spectra were collected on a J-810 spectropolarimeter (Jasco, Tokyo, Japan) employing 0.1 mm quartz water-jacketed cell. Peptide samples were prepared as described above and transferred to the cell at 37°C. The ellipticity in millidegrees were monitored at 216 nm as a function of time. After the kinetic measurement, a wavelength scan was recorded at 37°C using a 0.1 mm quartz jacketed cell. Peptide samples were prepared as described above and imaged by using 10× magnification on a 510 LSCM microscope (Zeiss, Jena, Germany).

Cell Culture and Confocal Microscopy. C3H10t1/2 cell growth conditions were 90% DMEM supplemented with 25 mM Hepes, 10% FBS, 5 mM L-glutamine, and 50 μg/ml Gantamacin at 37°C in 5% CO2. For confocal micrographs of hydrogels impregnated with prelabeled cells, a suspension of 1.5 × 106 cells per ml was incubated in a solution of 5 μM cell tracker green in DMEM for 45 min. After labeling, cells were washed three times with PBS and resuspended in DMEM at a concentration of 5 × 105 cells per ml. An equal volume of the cell suspension was added to a vial containing a solution of buffered peptide in 25 mM Hepes (pH 7.4); the resulting solution was immediately transferred to an eight-well borosilicate confocal plate and placed into the incubator at 37°C and 5% CO2. For shear-thinning experiments, the gel/cell constructs were prepared as above and immediately loaded into a 1-ml syringe equipped with a 20-gauge needle and allowed to undergo hydrogelation for 5 min before shear-thinning onto a confocal plate for imaging. For viability studies, the gel/cell constructs were prepared as above with unlabeled cells. Viability of encapsulated cells in 0.5 wt% hydrogels (before and after shear-thinning) was assessed by using a Live/Dead assay at 3 h after encapsulation. A stock solution containing both 1 μM calcein AM and 2 μM ethidium homodimer in DMEM were prepared according to the Live-Dead assay (Molecular Probes # L3224) package instructions, and 200 μl of this stock was added to each well. All gel/cell constructs were imaged by using 10× magnification on a 510 LSCM microscope (Zeiss, Jena, Germany).

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1. Methods
   a. Peptide Synthesis and Purification. Peptides were synthesized on RINK amide resin via an automated ABI 433A peptide synthesizer employing standard Fmoc-protocol and HCTU activation. The resulting dry resin-bound peptides were cleaved and side-chain deprotected for 2 hr under N₂ atmosphere using a TFA: thioanisole: ethanediithiol: anisole (90:5:3:2) cocktail. Filtration followed by ether precipitation afforded crude peptides that were purified by RP-HPLC (preparative Vydac C18 peptide/protein column). MAX1: Isocratic at 0% B for 2 min then a linear gradient from 0 to 18% B over 3 min, then 18 to 100 % B over 164 min. Peptide elutes at 29 min. MS (ESI) m/z: 1116.0 [(M+2H)²⁺, calcd 1115.9]. MAX8: Isocratic at 0% B for 2 min then a linear gradient from 0 to 25% B over 17 min, then 25 to 40% B over 30 min, then 40 to 100% B over 10 min. The peptide elutes at 31 min. MS (ESI) m/z: 1116.2 [(M+2H)²⁺, calcd 1116.5]. A flow rate of 8 mL/min was employed for preparative HPLC. Elutants for RP-HPLC consisted of solvent A (0.1 % TFA in water) and solvent B (90% acetonitrile, 10% water, 0.1% TFA). Lyophilized purified peptides were dissolved in water at 1 mg / mL & re-lyophilized twice.

![Analytical HPLC of purified MAX1](image1)

**Fig. 1.** (a) Analytical HPLC of purified MAX1 injected onto a vydac C18 column. The gradient is 0 to 100 %B over 100 min, peptide elutes at 27 min. (b) Mass spectra ESI (+) of purified MAX1.

![Analytical HPLC of purified MAX8](image2)

**Fig. 2.** (a) Analytical HPLC of purified MAX8 injected onto a vydac C18 column. The gradient is 0 to 100 %B over 100 min, peptide elutes at 31 min. (b) Mass spectra ESI (+) of purified MAX8.
b. Methods for: hydrogel formation in absence of cells, cell encapsulation within hydrogels, CD spectroscopy, oscillatory rheology, and TEM are given in the main text.

2. Reproducibility of Encapsulation and Shear Thin Delivery
In addition to the images shown in the manuscript, additional cell encapsulation and shear thin delivery experiments were performed with a separate lot of C3H10t1/2 cells and with HEP G2 cells (hepatocytes) showing reproducibility.

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<tr>
<th>Non-homogeneous encapsulation of C3H10t1/2 cells in MAX1</th>
<th>Homogeneous encapsulation of C3H10t1/2 cells in MAX8</th>
<th>Shear thin delivery showing homogeneous distribution of C3H10t1/2 cells in MAX8</th>
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**Fig. 3.** LSCM z-stack images (viewed along the y-axis through the gel, depth of field is 921.4 µm) showing 0.5 wt% MAX1 (a) and MAX8 (b) gel/cell constructs prepared directly in the confocal well with C3H10t1/2 cells and MAX8 (c) with cells prepared in a syringe and shear thin delivered to the confocal plate. (Scale bar, 100 µm.)

<table>
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<tr>
<th>Non-homogeneous encapsulation of HEP G2 cells in MAX1</th>
<th>Homogeneous encapsulation of HEP G2 cells in MAX8</th>
<th>Shear thin delivery showing homogeneous encapsulation of HEP G2 cells in MAX8</th>
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**Fig. 4.** LSCM z-stack images (viewed along the y-axis through the gel, depth of field is 921.4 µm) showing 0.5 wt% MAX1 (a) and MAX8 (b) gel/cell constructs prepared directly in the confocal well with HEP G2 cells and MAX8 (c) with cells prepared in a syringe and shear thin delivered to the confocal plate. (Scale bar, 100 µm.)
3. Dynamic Time Sweep and Dynamic Frequency Sweep Rheology of MAX1 and MAX8 Acellular Gels Showing both the Storage and Loss Modulus.

Fig. 5. Oscillatory rheology of 0.5 wt% MAX1 (squares) and MAX8 (triangles) hydrogels. MAX1 and MAX8 samples were prepared as stated in the experimental section (a) Dynamic time sweep (DTS) showing the onset of gelation at 37 °C monitoring the storage modulus, G’ (solid symbols) and loss modulus, G” (open symbols) as a function of time, frequency = 6 rad. sec⁻¹, strain = 0.2 %, gap = 0.5 mm. (b) Dynamic strain sweep performed immediately after the DTS at 37 °C monitoring the G’ and G” as a function of frequency, strain = 0.2 %, gap = 0.5 mm.

4. Live-Dead Control of Mesenchymal Stem Cells Encapsulated in MAX8 Hydrogels Prior to Shear Thin Delivery.

Fig. 6. LSCM z-stack image (viewed along the z-axis) showing a Live/Dead assay of an 0.5 wt% MAX8 gel/cell construct prepared directly in the confocal well at T=3 hr. Red = dead cells, green = alive cells. MAX8 gel/cell constructs were prepared as stated in the experimental section. Inset of an eye viewing down the z-axis enabling visualization of how the confocal image is displayed. (Scale bar, 100 µm.)