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The modulation of MSC integrin expression by RGD presentation

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Abstract

Biomaterials designed to mimic the intricate native extracellular matrix (ECM) can use a variety of techniques to control the behavior of encapsulated cells. Common methods include controlling the mechanical properties of the material, incorporating bioactive signals, spatially patterning bioactive signals, and controlling the time-release of bioactive signals. Further design parameters like bioactive signal distribution can be used to manipulate cell behavior. Efforts on clustering adhesion peptides have focused on seeding cells on top of a biomaterial. Here we report the effect of clustering the adhesion peptide RGD on mouse mesenchymal stem cells encapsulated inside three-dimensional hyaluronic acid hydrogels. The clustered bioactive signals resulted in significant differences in both cell spreading and integrin expression. These results indicate that signal RGD peptide clustering is an additional hydrogel design parameter can be used to influence and guide the behavior of encapsulated cells.

Keywords

Hydrogel; Hyaluronic acid; RGD clustering; Mesenchymal stem cells

1. Introduction

The microenvironment plays a crucial role in normal development by guiding stem-cell fate and tissue organization [1–9], but also contributes to pathological processes such as tumor progression and metastasis [1,3,4,10–19]. To be able to design materials that can be used to study stem cell fate decisions or as scaffolds for regenerative medicine, there needs to be a better understanding of how bioactive signal incorporation affects the embedded cells. For example, although vascular endothelial growth factor-165 (VEGF) has been routinely covalently immobilized to hydrogel scaffolds to enhance angiogenesis [20–23], the molecular consequences of this immobilization are not completely understood. We recently found that covalently bound VEGF is able to phosphorylate VEGFR-2 to the same extent as soluble VEGF in endothelial cells (EC), but that the mode of VEGF presentation alters the tyrosine residues that are phosphorylated, the time course of phosphorylation, and the resulting downstream signaling [24]. In this report, we studied how the presentation (clustered or homogenous) of the fibronectin derived peptide sequence, RGD, affected mesenchymal stem cell proliferation, spreading and integrin expression.

Hydrogels are a class of biomaterial scaffolds that exist as highly hydrated, cross-linked networks [25–27]. Their chemical and physical properties can be adjusted to create microenvironments suitable for cell proliferation and differentiation. Hydrogels can be constructed from natural materials like hyaluronic acid [28], collagen [29], fibrin [30], chitosan [31], and alginate [32]. Researchers have also used synthetic materials like poly(ethylene glycol) [33], and poly(vinyl alcohol) [34] to construct tissue engineering scaffolds. With a number of advantages and disadvantages to each type of material, the application can help choose the hydrogel system used by the researcher. Regardless of the system chosen, bioactive molecules must be incorporated into the hydrogel matrix to help direct cell behavior [35,36]. These molecules can consist of full-length extracellular matrix proteins like fibronectin, and laminin or small peptide sequences derived from these proteins like RGD, IKVAV and YIGSR [37–41].

Integrin binding connects the extracellular matrix to the cells and initiates signaling events that control cell behavior [42–45] and remodel the matrix [46,47]. Two-dimensional studies in hydrogel scaffolds have shown that RGD presentation at the nanoscale level influences cell spreading and motility [48–51], stem cell differentiation [52] and nanoparticle internalization [53], however, for cells seeded within the hydrogel scaffolds, only RGD total content has been shown to modulate cell motility, spreading and proliferation [28,54,55].

Herein we report on the effect of varying RGD presentation on mouse mesenchymal stem cell spreading, proliferation, and integrin expression for cells cultured inside matrix metalloproteinase degradable hyaluronic acid hydrogels. Hyaluronic acid is a nonsulfated glycosaminoglycan that exists in connective, epithelial, and neural tissue [56,57]. Its high biocompatibility and low immunogenicity highlight its potential as a biomaterial. We have previously shown that altering hyaluronic acid (HA) hydrogel parameters (HA weight%, crosslinker type, crosslinking ratio, RGD concentration) greatly affects encapsulated cell behavior [28]. The same hyaluronic acid hydrogel system was used, with the RGD clustering controlled by pre-reacting specific portions of the HA with the bioactive signal (Fig. 1). Mouse mesenchymal stem cells were encapsulated inside these hydrogels. These cells are multipotent and have shown the ability to differentiate into adipocytes, chondrocytes, and osteoblasts [58–61].

2. Materials and methods

2.1. Cells

Mouse mesenchymal stem cells (D1, CRL12424) were purchased from ATCC (Manassas, VA, USA) and cultured in Dulbecco's modified eagle's medium (DMEM, Sigma-Aldrich) with 10% bovine growth serum (BGS, Hyclone, Logan, UT) and 1% penicillin/streptomycin (Invitrogen, Grand Island, NY). They were cultured at 37 °C with 5% CO₂ using standard protocols.

2.2. Hyaluronic acid modification

Hyaluronic acid was functionalized with an acrylate group using a two-step synthesis as previously described [28]. HA (60,000 Da, Genzyme Corporation, Cambridge, MA) (2.0 g, 5.28 mmol) was dissolved in water and reacted with adipic dihydrazide (ADH, 18.0 g, 105.5 mmol) with 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC, 4.0 g, 20 mmol) at a pH of 4.75 overnight. The solution was purified via dialysis (8000 MWCO) in deionized water for 2 days. The hydrazide-modified hyaluronic acid (HA-ADH) was lyophilized and stored at –20 °C. HA-ADH (1.9 g) was re-suspended in 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) buffer (10 mM HEPES, 150 mM NaCl, 10 mM EDTA, pH 7.4) and reacted with N-acryloxysuccinimide (NHS-AC, 1.33 g,

4.4 mmol) overnight. After dialysis purification against deionized water for 2 days, the acrylated hyaluronic acid (HA-AC) was lyophilized. The product was analyzed with ¹H NMR (D₂O) and the degree of acrylation (16%) determined by dividing the multiplet peak at $\delta = 6.2$ (cis and trans acrylate hydrogens) by the singlet peak at $\delta = 1.6$ (singlet peak of acetyl methyl protons in HA).

2.3. Gelation

Lyophilized acrylated hyaluronic acid was dissolved in 0.3 M triethanolamine (TEOA) for 20 min at 37 °C. Ac-GCGYGRGDSPG-NH₂ adhesion peptide (RGD, Genscript, Piscataway, NJ) dissolved in 0.3 M TEOA was added to the appropriate amount of HA-AC and allowed to react for 20 min at 37 °C (Table 1). For example, in condition A3, 10 μ M of RGD was added to 21% of the total HA-AC required whereas condition A5 required the RGD to be reacted to only 1.4% of the total HA-AC. Full culture DMEM, mMSC's (5000 cells/ μ L final concentration), and the required amount of non-RGD functionalized HA-AC were then added. An aliquot of an MMP-degradable peptide crosslinker (Ac-GCRDGPQGIWGQDRCG-NH₂, Genscript, Piscataway, NJ) was dissolved in 0.3 M TEOA and added to the gel precursor solution. 10 μ L s of this solution was pipetted onto, and sandwiched between two Sigmacote (Sigma-Aldrich) functionalized glass coverslips and placed in an incubator for 30 min at 37 °C to gel. The degree of RGD clustering seen in Table 1 was calculated by dividing the moles of RGD used by the moles of HA that were reacted with the adhesion peptide.

2.4. Rheology

Gels without cells were made as described above and cut to size using an 8.0 mm biopsy punch. The modulus was measured with a plate-to-plate rheometer (Physica MCR 301, Anton Paar, Ashland, VA) using an 8 mm plate with a frequency range of 0.1–10 rad/s under a constant strain of 1% at 37 °C. An evaporation blocker system was used to keep the hydrogel from dehydrating during the test. For hydrogel degradation time course studies, gel A5 was made with cells and the modulus was measured at days 1, 4, and 7 after gelation.

2.5. Fixing/imaging

Gels were rinsed in 1 $\times$ PBS and fixed in 4% paraformaldehyde for 30 min at room temperature. Following a rinse in 1 $\times$ PBS, the gels were incubated in 0.1% Triton X-100 to permeate the cell membranes. Another 1 $\times$ PBS rinse was followed by a 90 min incubation in rhodamine phalloidin (Invitrogen) diluted 1:40 in a 1% BSA solution at room temperature in the dark. The gels were washed 3 times with 0.05% Tween-20 for 5 min prior to imaging with an inverted fluorescence microscope (Zeiss Axio Observer). 40 Z-stack slices were taken of each gel and the maximum intensity projection was taken following deconvolution image processing. Cell spreading was quantified by measuring the length of the longest cell dimension using the Axiovision software.

2.6. Cell proliferation

Cell proliferation was measured using the CyQUANT cell proliferation assay kit (Invitrogen). Gels were washed twice in 1 $\times$ PBS and frozen in a –80 freezer at days 1, 4, 7, and 10 after hydrogel gelation. After thawing the gels at room temperature, they were degraded by incubation in 1000 U/mL of collagenase I (Worthington, Lakewood, NJ) at 37 °C for 15 min. The cells were isolated by centrifuging the solution at 500 rcf $\times$ 5 min and resuspended in 200 μ L of the CyQUANT GR dye/cell-lysis buffer. After 5 min incubation, the fluorescence was read using a plate reader at 480 nm.

2.7. Flow cytometry

Gels at day 4 after gelation were rinsed in $1 \times$ PBS and degraded by incubation in 1000 U/mL of collagenase I at 37 °C for 15 min. The solution was spun at 500 rcf for 10 min and re-suspended in 1% BSA for 15 min to block nonspecific binding of antibodies. Each sample was incubated with one PE and one FITC-conjugated antibody for 30 min at a 1:40 dilution in 1% BSA on ice. The solution was centrifuged at 500 rcf for 10 min and re-suspended in 1% paraformaldehyde for FACS. Analysis was performed using a FACScan X and the data was analyzed using FLOWJO. Triplicates were done for each condition with 3000 events/sample. The data was gated such that the negative control had 5% positive events.

2.8. Statistics

Statistical analysis was performed using InStat (GraphPad, San Diego, CA). Data was analyzed using one way analysis of variance (ANOVA) test with a TukeyKramer post-test and a 95% confidence interval.

3. Results

3.1. Hydrogel synthesis and characterization

Hydrogel scaffolds were synthesized to contain different amounts of RGD ($A = 10 \mu\text{M}$, $B = 100 \mu\text{M}$ and $C = 1000 \mu\text{M}$) and different distributions from homogenous (1) to increasing degrees of clustering (2–5). Thus, gel A5 contains RGD that is more clustered than A3 and C3 has more RGD total content than A3 or B3. Table 1 details the hydrogel synthesis conditions and RGD content. Because the amount and distribution of RGD is changing within each hydrogel condition, we wanted to ensure that the mechanical properties of the gels were the same between conditions. Similar mechanical properties would ensure that any differences observed between gels A1–5, B1–B5, or C1–C5 were due to RGD presentation and not mechanical differences. Gels 1, 3, and 5 for each RGD concentration were made and the storage modulus measured using a plate-to-plate rheometer (Fig. 2A–B) with a constant strain of 1% between frequencies 0.1 and 10 Hz. Storage moduli between each concentration were not found to be statistically different ($p > 0.05$, Fig. 2B). However, increasing the amounts of RGD from 10 to 100 and 1000 μM did reduce the average storage modulus for those gels. Therefore, comparisons are not made between different RGD concentrations and only between different RGD presentations.

3.2. Cell spreading

The effect of RGD clustering on mMSC spreading was studied at three concentrations and five different presentations as described in Table 1. At days 1, 4, and 7 the cells were fixed and filamentous actin was stained with phalloidin. For gels containing 10 μM of RGD, increasing signal clustering resulted in a higher degree of cell spreading. The homogenous condition, A1, had an average cell length of $15.16 \pm 1.75 \mu\text{m}$ and was found to be statistically lower than the other 10 μM RGD conditions ($p < 0.001$). Gels A2–A4 had average cell lengths of 31.49 ± 8.66 , 27.33 ± 5.44 , and $36.50 \pm 5.74 \mu\text{m}$, respectively. Gel A3 was found to be statistically lower than A4 ($p < 0.001$). Gel A5 had the most spreading with cells averaging a length of $70.19 \pm 14.49 \mu\text{m}$. This condition was found to be statistically greater than gels A1–A4 ($p < 0.001$).

For gels with an RGD concentration of 100 μM , the measured cell lengths of B1–5 were 42.69 ± 10.22 , 48.55 ± 10.02 , 64.27 ± 13.23 , 37.69 ± 9.52 , and 32.18 ± 9.14 , respectively. B3 was found to be statistically greater than the other 4 conditions ($p < 0.001$). In addition, condition B2 was statistically different from B4 ($p < 0.05$) and B5 ($p < 0.001$).

Gels C1-5 containing 1000 μM of RGD had cell lengths of 31.98 ± 9.74 , 41.21 ± 12.61 , 73.66 ± 11.28 , 38.45 ± 12.27 , and 41.92 ± 17.47 , respectively. Gel C3 was found to be statistically higher than the other 4 conditions ($p < 0.001$) within this RGD concentration.

Cell spreading between A1–5, B1–5, and C1–5 can be compared through their degree of RGD clustering (RGD/HA molecule, Table 1). Gels A5, B3, and C3 had the highest degree of spreading for each RGD concentration, which corresponded to 1.8, 1.2 and 12 RGDs/HA molecule.

3.3. Proliferation

Proliferation within gels was measured by quantifying DNA content with a CyQUANT proliferation kit. Gels from each condition were washed twice with PBS and frozen at -80°C at days 1, 4, and 7. After thawing the gels, collagenase I was used to degrade the MMP-sensitive peptide used to crosslink the gels. Cells were isolated from the solution via centrifugation and lysed. For gels A1–A5 (RGD 10 μM , Fig. 3C) the DNA content rose each day except for day 10. No statistical difference between the conditions was found at each time point. DNA content rose in gels B1–B5 (RGD 100 μM , Fig. 4C) at each time point and there was no statistical difference between the conditions. Gels C1–C5 (RGD 1000 μM , Fig. 5C) had DNA content rise between each time point until day 10. No statistical difference between each condition was found at each time point.

3.4. Integrin expression

To see if changes in spreading due to RGD presentation altered integrin expression, cells were collected from gels A1, A3, and A5 at day 4. These conditions were shown to have significantly different amounts of cell spreading at this time point (Fig. 3B). We found that the RGD presentation did affect the expression of cell integrins (Fig. 6 and Fig. 7). Specifically, the hydrogel properties induced different expression levels in $\alpha 2$, $\alpha 3$, and $\beta 1$. The amount of cells that positively expressed $\alpha 2$ was significantly higher in A1 when compared to A3 ($p < 0.001$) and A5 ($p < 0.001$, Fig. 6B). Differences in $\alpha 3$ normalized mean fluorescence values were observed between A1–A3 ($p < 0.001$) and A1–A5 ($p < 0.001$, Fig. 6C). In addition, the percentage of $\alpha 3$ positively expressing cells was significantly lower in A1, when compared to A3 ($p < 0.001$) and A5 ($p < 0.001$, Fig. 6D). RGD presentation also stimulated differences in $\beta 1$ mean fluorescence levels between condition A1 and A5 ($p < 0.01$, Fig. 7A). The number of $\beta 1$ expressing cells also increased from gel A1 to A3 ($p < 0.01$) and A5 ($p < 0.001$, Fig. 7B). RGD presentation did not significantly affect the integrin expression levels of $\alpha 5$ (Fig. 6E, F), αV (Fig. 6G, H), and $\beta 3$ (Fig. 7C, D).

3.5. Time course of MSC marker

The MSC marker CD105 was used to determine whether the RGD clustering played a role in differentiating the mMSC's encapsulated in the gels. Cells from a flask were used as a “day 0” time point. Gels from A1, A3, and A5 were degraded at days 1, 4, and 7 and cells collected. The CD105 expression decreased with time with 87% of the cells expressing the marker at day 0 and only 17.3, 17.2 or 7.6 expressing the marker for A1, A3 and A5 conditions respectively. There were no statistical significant differences between conditions ($p > 0.05$).

3.6. Time course of gel degradation

The time course of gel degradation was investigated by encapsulating cells in A5 and measuring the storage modulus at days 1, 4, and 7 after gelation (Fig. 8C–D). The modulus

decreased at each time point as the protease's released by the cells broke down the structure of the hydrogel.

4. Discussion

Stem cells hold great promise due to their pluripotency [62–64]. Their fate is influenced by interactions with soluble factors, physical signals, other cells, and extracellular matrix mechanics. The effect of these factors on stem cell behavior must be studied and understood to unlock these cell's full potential. While there have been studies on the effect of mechanical properties on cell behavior, there has been less emphasis on the effect of bioactive signal presentation. Studies in 2-dimensional environments have shown the importance of signal clustering on cell behavior [50,51]. Here we study the effect of RGD peptide distribution on mouse mesenchymal stem cell behavior in a three-dimensional hyaluronic acid hydrogel.

RGD is a well-characterized peptide adhesion fragment derived from fibronectin that facilitates binding through the $\alpha 5 \beta 1$ integrin complex [38]. RGD peptide distribution in the hydrogel was controlled by pre-reacting it with specific percentages of the total acrylate modified hyaluronic acid (HA-AC, Table 1). To characterize the differences between each condition, the degree of RGD clustering was calculated. This is defined by the moles of RGD per mole of HA reacted with RGD or number of RGD per HA molecule (Table 1).

Before studying the effect of clustering on cell behavior, rheology was performed on all gel conditions. The storage modulus was found to be constant among each given RGD concentration (Fig. 2A–B). However, there were statistical differences when comparing gels with different amounts of RGD. This data allows us to directly compare gels with the same RGD concentration but not between different concentrations. Differences observed can be attributed to RGD presentation and not bulk mechanics of the hydrogel.

To investigate the time course of cell spreading, samples were fixed at days 1, 4, 7, and 10 after gelation and filamentous actin stained (Figs. 3A, 4A and 5A). The largest difference in cell spreading was observed at day 4 and was quantified for further analysis. For gels with 10 μM of RGD, the most spreading was observed in the most clustered sample (A5), which had 1.8 mmol of RGD/mmol HA-RGD (Fig. 3B). However, the most cell spreading in gels with 100 μM RGD occurred in the middle condition (B3, Fig. 4B). This condition had 1.2 mmol of RGD/mmol HA-RGD, which may indicate an optimal “degree of RGD clustering” for cell spreading in our hyaluronic acid hydrogels. The final RGD concentration tested, 1000 μM , had the highest degree of cell spreading in the middle condition (C3, Fig. 5B), which had 12 mmol of RGD/mmol HA-RGD. If the optimal “degree of RGD clustering” is near the clustering seen in gel conditions A5 and B3, gel condition C1 would be expected to produce more spreading than C3. A possible explanation for this discrepancy is that the sheer amount of RGD encountered by cells in conditions C1–C5 outweighs the effects of RGD clustering. Studies of cells seeded on top of patterned materials have also shown that bioactive signal distribution affects behavior [48–51]. After day 4, differences in cell spreading became less pronounced.

Cell spreading observations prompted us to investigate integrin expression to see if the clustering was affecting the cell surface receptors. We chose to look at gels A1, A3, and A5 at day 4 since these conditions had the biggest difference in cell spreading. We analyzed integrins that are known to be expressed in MSCs: $\alpha 2$, $\alpha 3$, $\alpha 5$, αV , $\beta 1$, and $\beta 3$ [45,65]. Differences in expression were found in multiple integrins. The percentage of cells positively expressing the $\alpha 2$ integrin was significantly higher in condition A1 than A3 and A5 (Fig. 6B). Both the percentage of cells expressing $\alpha 3$ and the degree of expression were

significantly lower in A1 than A3 and A5 (Fig. 6C–D). One of the beta subunits, $\beta 1$, also had different expression levels between conditions. There was a difference between the mean fluorescence levels in sample A1 vs. A5. The percentage of positively expressing cells was also lower in A1 when compared to A3 and A5 (Fig. 7A–B). Since integrin expression affects the signaling pathways that influence cell behavior and differentiation, we also tracked the expression time course of the mesenchymal stem cell marker, CD105, in gels A1, A3, and A5 (Fig. 8A–B). mMSC's taken from a tissue culture flask were used as a day 0 time point. Over the course of 7 days, CD105 dropped dramatically between all conditions. It was interesting to see that even though we had differences in spreading and integrin expression at day 4, there were not any differences in CD105 expression between conditions throughout the 7 day experiment.

Matrix mechanical properties have been shown to play a key role in determining cell fate [35,66]. Because of this, the time course of gel degradation was studied. Since our hydrogel is cross-linked with an MMP-degradable peptide sequence and the backbone is degradable by secreted hyaluronidases, the storage modulus decreases over time as the encapsulated mesenchymal stem cells release MMPs or hyaluronidases (Fig. 8C–D). This decrease in matrix stiffness could help explain why the RGD clustering is not able to control cell differentiation in our system. Because the hydrogel degrades, the differences due to bioactive signal clustering we observed in cell spreading and integrin expression at day 4 were not enough to influence changes in CD105 expression between conditions. The expression change seen is due to a combination of RGD clustering and mechanical changes, with mechanical signals weighing in more than bioactive signals [67]. To tease out the isolated role of bioactive signal clustering, we would need a system that keeps mechanical properties constant.

5. Conclusion

In this study, we investigated the effect of RGD bioactive signal clustering on the behavior of MSCs cultured in a three-dimensional hyaluronic acid hydrogel. Cell spreading and integrin expression were found to be affected by RGD clustering. The highest degree of spreading was found in the most clustered condition for the 10 μM RGD hydrogels (A5) but in the middle clustered condition for the 100 μM RGD hydrogels (B3). These results comparing different RGD concentrations with similar degrees of clustering may indicate a concentration independent “optimal clustering” parameter for cell spreading. Furthermore, expression of integrins $\alpha 2$, $\alpha 3$, and $\beta 1$ were affected by signal clustering. While our system was able to reduce the level of an MSC marker in the cells studied, it was not able to isolate the effect of the signal clustering from mechanical properties over the course of 7 days. Our results show that bioactive signal clustering has an effect on cells in three-dimension and is a biomaterial design parameter that can be manipulated to help control cell behavior.

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Fig. 1.

The hydrogel is composed of acrylated hyaluronic acid, MMP-degradable peptide crosslinker, and an RGD-motif containing peptide. RGD clustering is controlled by the amount of HA-AC pre-reacted with RGD. In the homogenous, or least clustered condition, the RGD is mixed with all of the HA-AC. The RGD is pre-reacted with specific percentages of the total HA-AC to create different degrees of clustering. This RGD functionalized HA-AC is mixed with un-functionalized HA-AC, if needed, peptide crosslinker and mouse mesenchymal stem cells to create the three-dimensional hydrogel.

Fig. 2. Mechanical characterization of hydrogel conditions. (A) Storage modulus of hydrogels was measured from 0.1 to 10 Hz. Three conditions from each RGD concentration were tested. (B) Mechanical properties within each RGD concentration were consistent, but increasing amounts of RGD lowered the storage modulus.

Fig. 3.

Cell spreading and proliferation for 10 μM hydrogels. (A) mMSC's within hydrogels containing 10 μM of RGD were stained with phalloidin at different time points. The most pronounced differences in spreading were observed at day 4. (B) The average cell length was quantified with the greatest spreading seen in the most clustered condition. (C) DNA quantification shows no significant difference between conditions at each time point.

Fig. 4. Cell spreading and proliferation for 100 μm hydrogels. (A) mMSC's cells within hydrogels containing 10 μM of RGD were stained with phalloidin at different time points. The most pronounced differences in spreading were observed at day 4. (B) The average cell length was quantified with the greatest spreading seen in the middle, B3, condition. (C) DNA quantification shows no significant difference between conditions at each time point.

Fig. 5. Cell spreading and proliferation for 1000 μm hydrogels. (A) mMSC's cells within hydrogels containing 1000 μm of RGD were stained with phalloidin at different time points. The most pronounced differences in spreading were observed at day 4. (B) The average cell length was quantified with the greatest spreading seen in the middle, C3, condition. (C) DNA quantification shows no significant difference between conditions at each time point.

Fig. 6. Integrin expression for subunits (A–B) $\alpha 2$, (C–D) $\alpha 3$, (E–F) $\alpha 5$, (G–H) αV was quantified for mouse mesenchymal stem cells cultured in gel conditions A1, A3, and A5 via FACS. Differences were found in the normalized mean expression for $\alpha 3$. Gel conditions also affected the number of cells positively expressing integrins $\alpha 2$ and $\alpha 3$.

Fig. 7. Integrin expression for subunits (A–B) $\beta 1$ and (C–D) $\beta 3$ for mouse mesenchymal stem cells cultured in gel conditions A1, A3, and A5 via FACS. Differences were observed in both the normalized mean fluorescence and percent of positively expressing cells for subunit $\beta 1$.

Fig. 8.

Time course of CD105 expression for mouse mesenchymal stem cells cultured inside hydrogels A1, A3, and A5 shows decreases across all three conditions for both the (A) normalized mean fluorescence and (B) the percent gated. (C–D) mechanical properties of hydrogel A5 was measured at days 1, 4, and 7. The storage modulus decreases over time as the cells release MMP's which breakdown the hydrogel structure.

Table 1

Hydrogel formulations tested contain 10, 100, or 1000 μM of RGD. Five different RGD distributions, from homogenous to increasing levels of clustering, were tested for each concentration. This distribution was controlled by functionalizing specific percentages of HA with the RGD peptide.

Hydrogel ID	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5
Total RGD (μM)	10	10	10	10	10	100	100	100	100	100	1000	1000	1000	1000	1000
% HA reacted w/RGD	100	42	21	4.2	1.4	100	42	21	4.2	1.4	100	42	21	4.2	1.4
RGD clustering (mmol RGD/mmol HA-RGD)	0.025	0.058	0.12	0.59	1.8	0.25	0.58	1.2	5.9	18	2.5	5.8	12	59	180