Fabrication of Smart Gels with Tunable Stiffness

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Abstract:

The differentiation of stem cells was influenced by many factors, one of which was the stiffness of the cellular microenvironment: soft substrates led to neuronal cells, intermediate stiffness substrates led to myogenic cells, and very rigid scaffolds lead to osteogenic cells [1]. To further understand the kinetics of stem cell differentiation, we designed degradable polyacrylamide hydrogels, which allowed us to change substrate stiffness during differentiation, and observe the effect of this change on the lineage specification of stem cells. With this purpose in mind, 8 wt% polyacrylamide gels composed of permanent *N*,*N*′-methylenebisacrylamide (BIS) and degradable *N*,*N*′-bis-(acryloyl)cystamine (BAC) cross-linkers were constructed. The shear modulus of these gels can be changed from ~ 10 kPa to ~ 400 Pa. Under normal conditions, these gels would lead to osteogenic and neuronal differentiation, respectively. This hydrogel was found to be degraded in a time span that maintained continuous cell proliferation with the biocompatible chemical tris(2-carboxyethyl)phosphine hydrochloride (TCEP) in Dulbecco's modified eagle's medium (DMEM). Since stem cells are known to commit to their final cell type within a week, using these gels will allow us to test whether the time point at which the scaffold is degraded effects the final lineage specification of the cells.

Experimental Procedure:

For our experiments, we made polyacrylamide gels and varied the amount of degradable and permanent crosslinkers. We used an 8 wt% polyacrylamide, 0.46 wt% BAC, 0.03 wt% Bis solution; this ratio was chosen to mimic a stiff, Bis only, gel (8 wt% acrylamide, 0.3 wt% Bis) predegradation and a soft gel (8 wt% acrylamide, 0.03 wt% Bis) after being degraded. By laying a 12 mm diameter coverslip on the solution and polymerizing the gel at 65°C, we fabricated degradable gels that were about 100 μm thick. The gels were then treated with a Sulfo-SANPAH solution followed by a 0.1 mg/ml collagen solution. The addition of collagen to the surface allowed the cells to adhere to the gel.

For initial tests of the degradable gels, HF fibroblasts of the BJ line are cultured on the gels and after about five hours, imaged as the gels degraded in a 10 mM TCEP (pH 7) solution over a period of ten hours. The Young's modulus of the degradable gel was measured on a Squisher device, or modified atomic force microscope (AFM), while the shear modulus was measured on a rheometer.

Results and Conclusions:

We tested several compounds to degrade the gel for both biocompatibility and effective degradation. We defined a compound as being biocompatible if cells continued to proliferate after exposure. To measure degradation, we simply allowed the gel to swell and degrade, and measured the time it took to complete the process. A 10 mM TCEP at a pH of 7 that was diluted in DMEM was determined to be the reducing agent that was biocompatible and able to degrade the gel on a desirable timescale.

Tested Chemicals for Degradation	Degrades gel	Biocompatible: in media	in DMEM
Dithiothreitol = DTT	✓	✗	
Glutathione = GSH	✗	✓	
GSH in HEPES buffer	✗	✗	
L-Cystine	✗	✗	
Hydrazine	✓	✗	
L–Ascorbic acid (Vitamin C)	✓	✗	
2-Phospho-L-ascorbic acid	✗	✗	
Bisulfite	✗	✗	
Tris(2-carboxyethyl) phosphine hydrochloride = TCEP (ph 7)	✓	✗	✓
Tris(2-carboxyethyl) phosphine hydrochloride = TCEP (ph 3)	✓	✗	✗

Figure 1: The chemicals that were tested to degrade the hydrogels composed of 8% polyacrylamide, 0.46% BAC, 0.03% Bis solution.

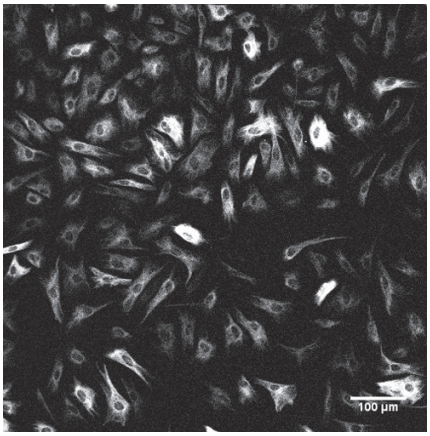

Figure 2: Fibroblasts cultured on the degradable gel before the degradation process.

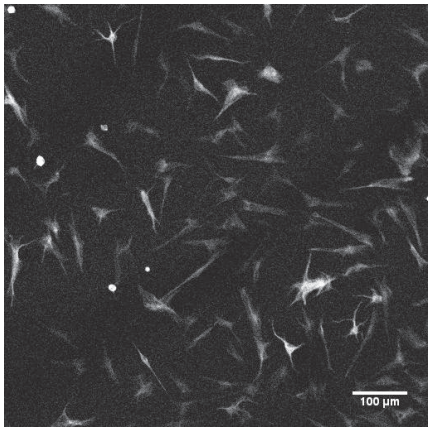

Figure 3: Fibroblasts after a 12 hour 10 mM TCEP treatment to degrade the gel.

Using a rheometer, we measured the change in the gel stiffness during polymerization as the temperature increased to 65°C. Our degradable gels had a shear modulus of the same order as a 0.3% Bis gel (stiff gel) and a degraded gel was estimated to have a shear modulus similar to a 0.03% Bis gel (soft gel) and should favor osteogenic and neurogenic differentiation, respectively.

To confirm these measurements, we used a "Squisher" device. By compressing an indenter into the gel by a known distance, we were able to measure the Young's modulus of the gel and determined that 100 mM and 10 mM TCEP conditions had the same degradation rate during a 12.5 hour degradation process. The kinetics of the degradation process for the degradable gel between 100 mM and 10 mM TCEP remained the same despite changes in the pH of the TCEP conditions.

To measure the effect of degradation on cells, we observed the effect of an ~ ten-fold decrease in the stiffness of our degradable gel on fibroblasts and found that the cell area decreased after the gel is degraded. A morphology change between cells that were cultured on our degradable gel before the degradation process and those cultured on our gels after the degradation process was also noted. Those cultured on the initially stiff gels were spread out and after the TCEP treatment; they remained spread out, while cells cultured on pre-degraded, soft gels had a rounder morphology.

In conclusion, 8% polyacrylamide 0.46% BAC 0.03% Bis gels can be made to have a ten-fold decrease in shear modulus after degradation. A 10 mM TCEP in DMEM was determined to be a biocompatible reducing agent that is able to degrade the gels on a desired timescale. Preliminary results showed that TCEP was biocompatible with and didn't interfere with the differentiation of stem cells, however, further experiments are required to indicate statistical significance.

Future Work:

With applications in tissue engineering, future works for this project include culturing Mesenchymal stem cells on degradable gels to study the differentiation on different stiffness scaffolds as well as finding a biocompatible chemical that reversibly degrades the gels.

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References:

[1] Discher, D.E., Janmey, P., and Wang, Y.L. Tissue cells feel and respond to the stiffness of their substrate. Science. 310(5751):1139-43;2005.

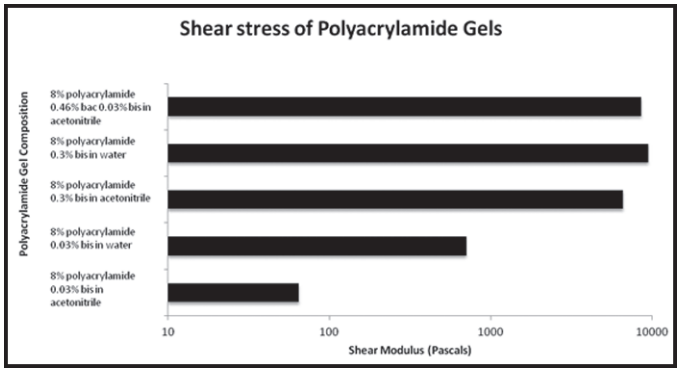

Figure 4: Comparison of the shear modulus of polyacrylamide gels of different cross-linker compositions with the degradable gel being the 8% polyacrylamide 0.46% BAC 0.03% Bis gel.