Modified PEGDA Hydrogels to Promote Mesenchymal Stem Cell Adhesion *In Vitro*

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Statement of Purpose: Mesenchymal stem cells (MSCs) have great therapeutic potential as they are able to differentiate into osteocytes, adipocytes, and chondrocytes. However, MSCs represent a fraction of the cells present in the bone marrow, with an approximate population density of .0001% of all nucleated cells in bone marrow (Augello A, et al. Eur Cell Mater. 2010;20:121-33.) Current methods of isolation, such as plastic adherence have limitations including loss of MSCs and unwanted expansion of non-MSCs (Niehage C, et al. PLoS One. 2011;6(5): e20399.) Once expanded, MSCs are often transferred onto new materials for *in vitro* studies. The purpose of this work is to create a unique material specific to MSCs that can capture and culture MSCs for future *in vitro* studies. Specifically, this study will investigate the effect of poly(ethylene glycol) diacrylate (PEGDA) hydrogels that have been modified on the surface with acryloyl-PEG-NH_2. The addition of amine groups at the surface will result in a charged surface, similar to tissue culture polystyrene (Davis JM. Animal Cell Culture: Essential Methods. 2011; John Wiley & Sons.) Inclusion of amines will confirm our fabrication methods and allow the investigation of substrate stiffness. We hypothesize that modifying the stiffness and surface of the PEGDA hydrogels will increase the adhesion of MSCs.

Methods: PEGDA hydrogels were created using free radical polymerization (Beamish JA, et al. J Biomed Mater Res A. 2010; 92(2): 441-50.) Hydrogels were tested at two concentrations; soft (5% PEGDA, E = 0.18kPa) or stiff (20% PEGDA, E = 1.9kPa). Solutions containing 5.0 or 20.0mg PEGDA/100µl PBS were used to vary stiffness. 10 µL of a photoinitiator solution containing 3mg/mL of 2,2-dimethyl-2-phenyl-aceptophenone in N-vinylpyrrolidone was added per mL of PEGDA solution. The combined PEGDA and photoinitiator solution was poured into a custom mold and exposed to long wavelength UV light for 5 minutes to ensure complete crosslinking. For surface modified gels, 25 µL of .012g/mL acryloyl-PEG-NH2 (Nanocs, Inc., Boston, MA) solution was added to the bottom of the mold, followed by the addition of the PEGDA and photoinitiator mixture. Hydrogels were crosslinked for 5 minutes. The presence of amine groups was confirmed by submerging hydrogels in a 1M ninhydrin solution and heating at 80° C. Once exposed to ninhydrin, attached amines resulted in a deep purple color. The chemistry and attachment method was also tested using acryloyl-PEG-FITC (Nanocs), which resulted in fluorescent gels when viewed with a fluorescent microscope. For cell culture experiments, hydrogels were washed in PBS for 48h and UV sterilized. Live/dead viability staining was used to test the viability and morphology of MSCs seeded on all hydrogels. To evaluate cell adhesion, a centrifugation assay (Fig. 1) was completed (Kaplan, DS, et al. Tissue Eng Part C Methods, 2012; 18(7):537-44.) MSCs were incubated for 1h in wells that contained modified or unmodified PEGDA hydrogels. After incubation, images were taken for cell count analysis. The well plate was inverted and centrifuged at 350xg, followed by imaging to determine the percent of adherent cells. Data was analyzed using one-way analysis of variance and Tukey's multiple-comparison test to determine statistical differences between hydrogels.

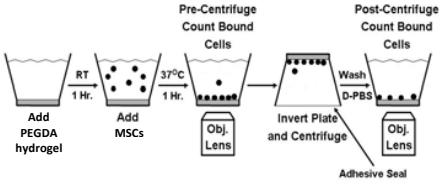

Figure 1. Centrifugation assay schematic.

Results: Surface modification was confirmed using a 1M ninhydrin stain. Both modified and unmodified gels were seeded with MSCs and the viability and spreading of cells were observed. Results indicated that a higher number of cells remained on the surface of stiffer gels compared to softer gels. Additionally, MSCs appeared to cluster and spread on the surface of the 20% modified gels, indicating attachment of the cells to the hydrogel surface. Cell clustering could be the result of an uneven amine group distribution on the hydrogel surface, resulting in increased cell attachment only at the location of amine groups. Cells that remained on the surface of stiff unmodified gels were much fewer in number, evenly distributed, and exhibited a rounded morphology. Qualitative findings were confirmed by the centrifugation adhesion assay. Modified hydrogels were found to have increased adhesion over unmodified gels.

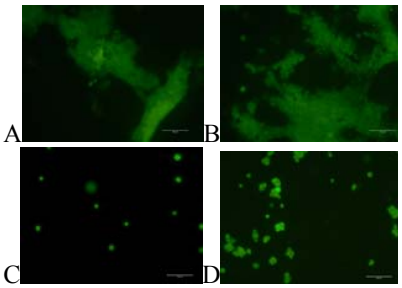

Figure 2. (A), (B) attachment and spreading on 20% modified gels. (C) Minimal attachment on 5% modified gels. (D) MSCs exhibiting spherical morphology on 20% unmodified gels. All scale bars = 100µm

Conclusions: Results showed that PEGDA hydrogels modified at the surface to contain covalently bound amines resulted in increased MSC adhesion. Adhesion was also increased with increasing hydrogel stiffness, indicating that MSCs prefer a stiffer substrate for cell attachment and proliferation. These results will be used in future studies to investigate how the incorporation of MSC specific proteins at the PEGDA surface influences the adhesion, proliferation, and differentiation of MSCs.