On-Demand Retrieval and Evaluation of MSCs Encapsulated in Enzymatically-Degradable PEG-Based Hydrogels

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Introduction: Matrix metalloproteinases (MMPs) are enzymes expressed by a variety of cell types to remodel their extracellular matrix. The inclusion of MMP-sensitive sequences in poly(ethylene glycol) (PEG)-based hydrogel systems allows for localized hydrogel degradation, enabling behaviors such as cell spreading and migration. Furthermore, cells can be recovered from gels post-culture through exogenous addition of enzymes like collagenase. Thus, this system may be used to differentiate stem cells in these gels via exposure to external stimuli, followed by on-demand cell recovery for delivery to injury sites. As a first step, we investigated the possible effects of cell spreading on stem cell markers by encapsulating human mesenchymal stem cells (hMSCs) in MMP-cleavable PEG using two different peptides known to exhibit different degradation kinetics. Through 7d, we analyzed cell morphology and the expression of putative MSC cell surface markers using flow cytometry following enzymatic gel degradation.

Methods: To create MMP-sensitive Acrl-PEG-peptide-PEG-Acrl products (referred to as LGPA and VPMSMR), the peptides GGGLGPAGGK and GGVPMSMRGGGK [1,2] were each reacted at a 1:2.2 molar ratio with Acrl-PEG-SVA (MW ~3,400) for 3h then dialyzed for 2d and lyophilized. A 50:50 wt% mixture of oligo[poly(ethylene glycol) fumarate] (OPF 10K, nominal M_n 10,000) and PEG-diacrylate (PEG-DA, MW ~3,400) was used as a non-degradable control. Acrl-PEG-GRGDS (reacted as above) at 1 µmol RGD/g gel was added to all gels to enable cell adhesion. Total polymer was 10% of initial total gel weight. 0.05 wt% D2959 in PBS was added to enable photocrosslinking under UV light (365 nm, 10 mW/cm^2, 10 min) in 6x1 mm circular Teflon molds. Human MSCs (passage 3; Texas A&M) were seeded in gels at 10x10^6 cells/mL. All constructs were then placed in low glucose DMEM with 10% fetal bovine serum and 1% antibiotic/ antimycotic. To evaluate cell spreading, all gel types were stained at 1d, 3d, and 7d with LIVE/DEAD dye and imaged using confocal microscopy (n=3). Images were taken 100 µm below the gel surface and live cells were analyzed for MSC circularity using ImageJ. Circularity is defined as $4\pi A/P^2$, where A and P are the cell area and perimeter, respectively; 1.0 is a perfect circle. For cell degradation and flow cytometry evaluation, each gel was placed in culture media containing 4 mg/mL bacterial collagenase type II. Gels (n=3-4) were placed on a shaker plate in an incubator until fully degraded (≤ 30 min). Retrieved cells were rinsed in phosphate buffered saline (PBS) and fixed in 10% buffered formalin. For flow cytometry, mouse anti-human CD73-APC, CD90-FITC, CD105-PerCP-Cy5.5, and CD146-PE were used to stain samples per manufacturer's recommendations. Samples were analyzed using an Accuri C6 flow cytometer with standard settings. Cells cultured on tissue culture plastic and then exposed to collagenase solution for 15 min served as an additional control. All data were statistically compared using two-way ANOVA and Tukey's HSD test.

Results and Discussion: MSCs were largely viable in all gel types. MSCs in OPF gels remained circular over time, but MSCs spread in LGPA gels, with significant differences in circularity at 7d. MSCs in VPMSMR gels were visibly spread at day 1; circularity was significantly lower than all other gels at 7d (Figure 1).

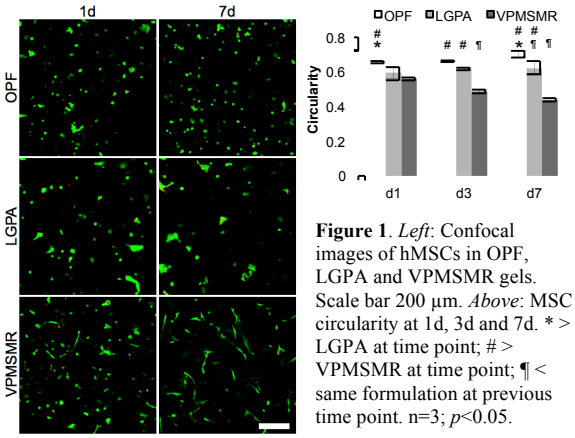

Figure 1. *Left*: Confocal images of hMSCs in OPF, LGPA and VPMSMR gels. Scale bar 200 µm. *Above*: MSC circularity at 1d, 3d and 7d. * > LGPA at time point; # > VPMSMR at time point; ¶ < same formulation at previous time point. n=3; p<0.05.

From flow cytometry results after degradation of MMP-cleavable gels, shifts in staining fluorescence translated to significant differences between all encapsulated cells vs. plated controls (data not shown). However, no differences were noted between peptide types or time points, and all samples expressed all four markers ~90% (Figure 2).

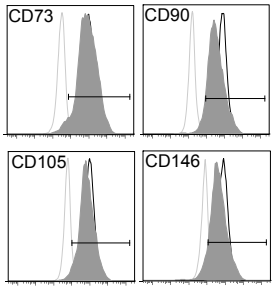

Figure 2. Flow cytometry plots showing representative samples of hMSCs isolated from either LGPA or VPMSMR hydrogels. Light gray represents mouse IgG fluorescent controls; black line represents hMSCs in collagenase solution for 15 min, then fixed and analyzed. Gray is a representative gel sample. A minimum of 5000 events were gated for each sample (n=3-4).

These studies indicate that cell morphology can be modulated by the inclusion of MMP-cleavable peptide sequences, dependent on the degradation kinetics of the particular peptide used. These sequences also enable facile cell retrieval through enzymatic gel degradation, allowing for analysis not possible in non-degradable gels. Flow cytometry results indicate that cell morphology alone does not effect meaningful changes in MSC-related cell surface markers. However, these results provide a baseline that will inform future studies examining the effect of additional external stimuli on MSC differentiation.

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References: [1] Patterson J, Biomaterials 2010 31:7836-46. [2] Gobin AS, FASEB J 2002 16:751-3.

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