Growth and Differentiation of Human Mesenchymal Stem Cells in Polymer-Peptide Hydrogels that Undergo Cell-Mediated Degradation

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Statement of Purpose: Mesenchymal stem cells (MSCs) are bone marrow derived multipotent cells capable of differentiating into a wide array of cells types, including chondrocytes and osteoblasts[1]. Clinical use of autologous MSCs for repair of cartilage and bone defects is attractive due to the ease of MSC isolation, expansion and differentiation. Creating a biomaterial scaffold for MSC culture increases the clinical versatility of MSCs by enhancing cell localization, increasing cell survival and promoting deposition of native extracellular matrix. Poly(ethylene glycol) (PEG) based hydrogels are inert, cytocompatible platforms that can be tuned with biomimetic cues to promote cell growth and differentiation. While previously characterized gel platforms are adequate for differentiating MSCs into chondrocytes and osteoblast[2,3], these hydrogels lack degradation capabilities that are essential for cell growth and spreading, as well as eventual device removal. Here we utilize recently developed thiol-ene PEG-peptide hydrogels to create a MMP-degradable three-dimensional culture environment for growth, differentiation and spread of MSCs.

Methods: $5x10^6$ human MSCs/ml were mixed in a monomer solution containing 8wt% 4-arm PEG-norbornene (MW. 20kDa), 15 mM matrix metalloproteinase cleavage peptide (MMP) (KCGPQGIWGQCK), 1 mM cell adhesive integrin ligand CRGDS peptide and 0.05% photoinitiator[4]. Hydrogels were formed via a radical-mediated step-growth cysteine-norbornene (thiol-ene) photopolymerization, creating a three-dimensional homogeneous PEG-peptide network that is proteolytically degradable by cell-secreted MMPs[5]. MSCs morphology was observed by labeling with Calcein AM (Invitrogen). Replicating DNA was labeled using the Click-iT EdU cell labeling kit (Invitrogen). Immunostaining was performed using mouse anti-CD105 (AbNova) and rabbit anti-collagen II (AbCam).

Results: MSCs encapsulated in MMP-degradable PEG-peptide hydrogels maintain high viability for up to 15 days as demonstrated by metabolic activity and Live/Dead cell staining. MMP-degradable PEG-peptide hydrogels provide a distinct advantage over non-degradable gels in that cells have the potential to spread and proliferate. The ability of MSCs to spread in response to TGF-β1, a known inducer of MSC migration, was tested by seeding cells on PEG-peptide hydrogels and allowing cells to spread for 5 days (Figure 1A). MSCs seeded on gels polymerized with TGF-β1 spread farther than control cells. Additionally, proliferation of MSCs in PEG-peptide hydrogels was also observed by labeling replicating DNA (Figure 1B). To demonstrate the efficacy of this platform for differentiating MSCs, cell-laden gels were cultured in chondrogenic media. Under these conditions, cells survive for up to 21 days and production of chondrogenic proteins was observed after 14 days (Figure 2). Similarly, MSCs survive and secrete a mineralized matrix when cultured in osteogenic media (Figure 3).

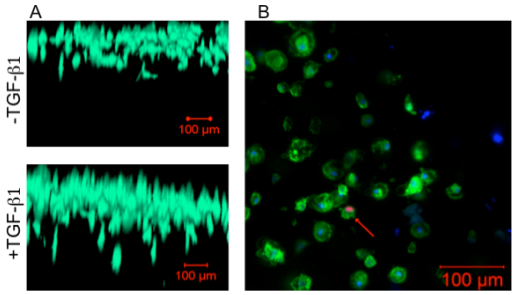

Figure 1. (A) MSCs spread into degradable PEG-peptide hydrogels following stimulation with TGF-β1. Projection is along the Z-axis. Live cells were labeled (Green). (B) MSCs proliferate in degradable PEG-peptide hydrogels. Cells were labeled for replicating DNA (Red), undifferentiated MSCs (CD105)(Green) and DNA (Blue). Arrow indicates proliferating cell.

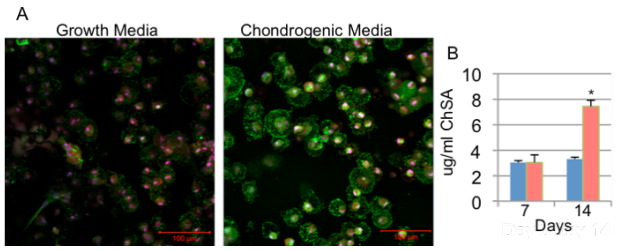

Figure 2. (A) Encapsulated MSCs produce Collagen II when cultured in chondrogenic media for 14 days. Hydrogels were immunostained for DNA (Blue), CD105 (Red) and Collagen II (Green). (B) MSCs produce glycosaminoglycans when cultured in chondrogenic media. Cell-laden hydrogels were assessed by DMMB assay for glycosaminoglycan content. * $p<0.05$.

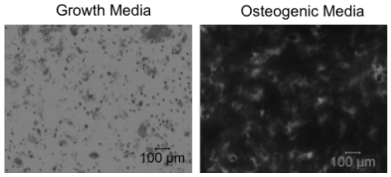

Figure 3. MSCs produce a mineralized matrix when cultured in osteogenic media. Cell-laden hydrogels were stained with Alizarin Red mineralization stain after 21 days in culture.

Conclusions: MMP-degradable thiol-ene PEG-peptide hydrogels were developed as a robust 3D culture platform for promoting the survival, spreading, proliferation and differentiation of MSCs. This platform can be used for encapsulation of MSCs in situations where degradation of the material, cell spreading and migration or cell proliferation are desired.

References: [1]Pittenger et al., *Science* 284(1999):143. [2]Salinas et al., *Tissue Eng* 13(2007):1025. [3]Nuttelman et al., *J Biomed Mater Res A* 68(2004):773. [4]Fairbanks et al., *Biomaterials* 30(2009):6702. [5]Fairbanks et al., *Adv Mater* (2009) In Press.