**Identifying targets for peptide-based osteogenic induction for osteochondral tissue engineering**

Aaron H. Aziz and Stephanie J. Bryant.
University of Colorado at Boulder.

**Statement of Purpose:** The overall objective of this project is to design a hydrogel (with appropriate mechanical properties, chemistry, bioactivity, and degradation) that utilizes the differentiation of hMSCs to restore biological function to the osteochondral interface. This interface is a complex 3D junction between two distinct tissues. As a result, both chondrogenesis and osteogenesis of hMSCs must be attained in a single multilayer construct. The most common approach to promote differentiation of hMSCs is through the use of soluble inducers. While soluble inducers can be delivered locally to control differentiation[1] our approach aims to utilize insoluble cues (e.g., peptides) to target and signal hMSCs. In this study, we focus on identifying key peptides that enhance osteogenesis for applications in osteochondral tissue engineering.

**Methods:** HMSCs (Texas A&M Health Science Center) were seeded at a density of 16,000 cells/cm$^2$ in either osteogenic media or control media, which was changed thrice weekly up to three weeks. At day 21, cells were washed, fixed and stained Alizarin Red S or were lysed, and DNA and enzyme collected. Alkaline phosphatase activity was measured in triplicate by p-nitrophenyl phosphate assay and normalized to DNA content (Quant-iT PicoGreen dsDNA Assay kit). At prescribed time points, immuno-cytochemistry was performed and images acquired by confocal microscopy. To develop hydrogels, a macromer solution consisting of 15% or 20% (w/w) 8-arm PEG-norbornene, PEG-dithiol at 1:1 thiol:ene ratio, and 0.05% (w/w) photoinitiator (I2959) in phosphate buffered saline was photopolymerized via ultraviolet light (10 min, 5-10 mW/cm$^2$, 352 nm). A second layer was then added and subsequently polymerized.

**Results:** A study was done to identify potential targets to stimulate osteogenic differentiation. Specifically, two classes of proteins were examined, integrins due to their involvement in intracellular cell signaling[2] and MMPs to incorporate cell-induced enzymatic remodeling[3]. From an initial screening, gene expression (not shown) along with previous research supported targeting two proteins in particular, ITGA5 which was shown to enhance ERK1/2-MAPKs and PI3K signaling to promote osteogenesis[4] and MMP14 determined to regulate Runx2 expression and support osteogenic differentiation of hMSCs[5]. To probe the presence of these proteins as targets for peptide signaling, a 21-day study following standard 2D osteogenic differentiation of hMSCs was conducted. Osteogenic differentiation was confirmed by mineral deposition and alkaline phosphatase activity (Fig 1A&B). The presence of protein targets was compared against hMSCs in control media and an osteoblast cell line (CRL-11372). Immuno-fluorescence shows strong staining for ITGA5 and MMP-14 in both osteogenic and basal media throughout the 21-day study (images for day 7, 10, and 14 time points are not shown). Staining was also performed on an osteoblast cell line, confirming presence of proteins in matured osteoblasts (data not shown).

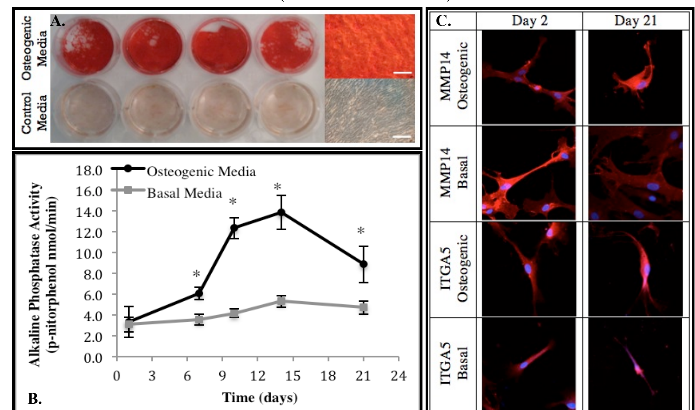


**Figure 1:** *A*. Mineral deposition (red) using Alizarin Red S of hMSCs after a 21-day differentiation in respective media. Scale bars represent 500 µm. *B*. Alkaline phosphatase activity time course of hMSC cultures grown respective media. Results represent the mean ± SD of triplicate cultures. * $p<0.05$ indicates significant difference from control. *C*. Immuno-cytochemistry of ITGA5 and MMP-14 samples at days 2 and 21.

In addition, the second part of this work was to establish a bilayer hydrogel system that could be used for testing these peptides in the boney layer of an osteochondral construct. Preliminary studies confirm the formation of multilayer hydrogels from two different formulations of PEG thiol-ene macromere solutions resulting in a relatively thin interface of ~40µm (Fig. 2).

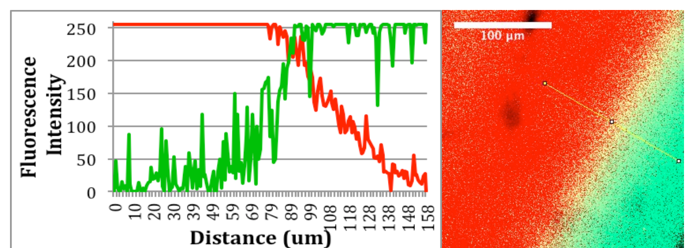


**Figure 2:** Rhodamine and fluorescein-labeled dyes in first and second layers, (20% and 15% wt/wt PEG hydrogels) respectively. Scale bar represents 100 µm.

**Conclusions:** Our findings suggest that ITGA5 and MMP14 may be potential peptide targets for promoting cell attachment and degradation, respectively, as they are present during all possible cellular fates (undifferentiated hMSCs, differentiated hMSCs and mature osteoblasts). Studies are underway to create bilayer hydrogels with these peptides and assess their ability to enhance differentiation in the absence of soluble inducers.

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**References:** 1) Kinard LA. Pharm Res. 2013;30:2332-2343. 2) Marie PJ. Nat Rev Endocrinol. 2013;9:288-295. 3) Patterson J. Biomaterials. 2010;31:7836-7845. 4) Hamidouche Z. Proc Natl Acad Sci. 2009;106:18587-18591. 5) Lu C. Blood. 2010;115:221-229.


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