Using Bioactive Gradients to Measure the Effect of Immobilized Peptide Density on Human Marrow Stromal Cell Proliferation and Differentiation

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Statement of Purpose: Enhancing design of tissue engineered scaffolds for directing bone regeneration *in vivo* requires a comprehensive understanding of cell interactions with immobilized bioactive molecules. Toward this goal, understanding the effect of immobilized bioactive molecule density on cell proliferation and osteogenic differentiation is a key step. Previous research has not focused on the effect of bioactive molecule density due to the amount of samples required per experiment. But, the recent development of gradient substrates for click bifunctionalization provides the technology to measure cell response to multiple immobilized bioactive molecule densities on a single substrate surface.[1] In the current study, this technology was used to measure human marrow stromal cell (hMSC) proliferation and differentiation in response to bioactive peptides. Pluripotent hMSCs are directed toward osteogenesis *in vivo* through extracellular interactions with matrix proteins and growth factors. Therefore this study focused on measuring cell response to peptides derived from these proteins and growth factors. (Table 1)

Table 1. Peptides coupled to the gradient

Peptide	Protein
KRSR	Bone Sialoprotein
RGD	Collagen I/Fibronectin
BMP	Bone Morphogenic protein-2

Methods: *Substrate fabrication.* All peptides were synthesized with an Apex 396 peptide synthesizer (Aapptec, Louisville, KY) using standard solid phase Fmoc chemistry and functionalized as described previously.[1] To synthesize gradients, an octyldimethyl-chlorosilane self assembled monolayer (SAM) was formed on glass by vapor deposition for 48 h. SAM substrates were exposed to ultraviolet oxidized (UVO) treatment for increasing lengths of time (0 s to 120 s) over a 40 mm distance and further derivatized with a bi-functional ethylene oxide linker as previously described.[1] Peptides were reacted with linker gradients via click chemistry. Gradients were verified with water and methylene iodide contact angles, and x-ray photoelectron spectroscopy. The standard uncertainty of contact angle measurements at each point along the gradient was determined by the standard deviation of three independent measurements on three substrates prepared under identical conditions. *Cell Culture and Characterization:* hMSCs (Tulane Center for Gene Therapy, New Orleans, LA) were cultured in αMEM media (Gibco) supplemented with 16.5 % (by volume) fetal bovine serum, 2 mmol/L L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin and incubated at 37 ºC with 5 % CO_2. Cells were seeded on gradient substrates (25 cells/mm^2) and cultured for 3 d, 7 d, or 14 d. Cell proliferation and differentiation on the gradients were measured using fluorescent microscopy and quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR). The standard uncertainty of cell density measurements at each point along the gradient was taken to be the standard error of the mean.

Results: Water contact angles on SAM, SAM-linker, and SAM-linker-peptide surfaces decreased from 100 ºC to 50 ºC over the length of the substrate (0 mm to 40 mm) and surface energies increased on average ≈ 400 dynes/cm, ranging from 600 dynes/cm to 1400 dynes/cm. (Fig. 1)

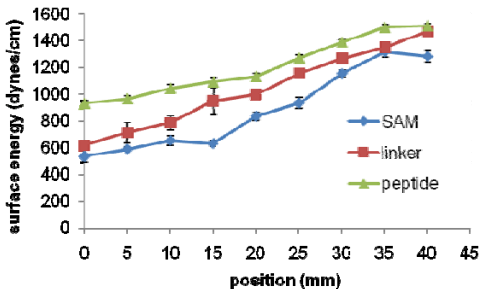

Figure 1. Surface energies of gradient substrates

At 3 d, cell density of hMSCs was highest on the hydrophilic end of the gradient (35 mm) with the peptide combination (50 % RGD peptide and 50 % BMP-2) inducing the most cell proliferation. By day 7, the RGD/BMP-2 peptide combination still promoted the most cell proliferation relative to other peptides, but the SAM gradient alone showed comparable influence on cell proliferation. By day 14, the cells reached confluency and peptides did not impact cell proliferation further. (Fig. 2) Preliminary RT-PCR results show increased bone sialoprotein mRNA in cells seeded on gradients with BMP/RGD or KRSR peptide gradients by day 7 suggesting the onset of osteogenic differentiation.

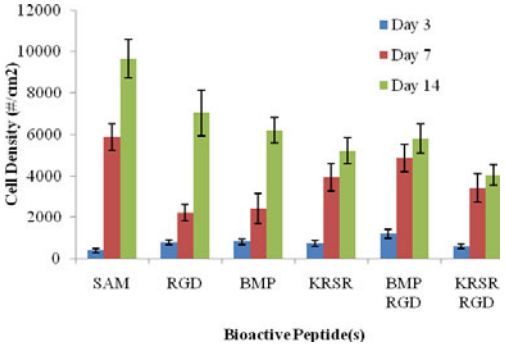

Figure 2. Cell density of hMSC on gradients at 35 mm

Conclusions: Synthesis of peptide gradient surfaces with "click" chemistry enabled the measurement of hMSC response to bioactive peptide densities. A combination of immobilized RGD and BMP-2 peptide promotes hMSC proliferation and differentiation at higher densities.

References:

1. Gallant, N.D., et al., *Adv Mater*, **2007**. 19(7): p. 965.