

## Stem Cell Differentiation on Adipocyte-Based Protein Micropatterns

Anita Shukla<sup>1</sup>, Jennifer L. West<sup>2</sup>

<sup>1</sup>School of Engineering, Brown University, <sup>2</sup>Department of Biomedical Engineering, Duke University

**Statement of Purpose:** Stem cells are of great interest for regenerative medicine. There is significant interest in better understanding and optimizing differentiation of these cells using physical and mechanical stimuli as compared to differentiation based solely on chemical cues. It has been shown that properties including substrate stiffness (Engler AJ. *Cell*. 2006;126:677-689), cell shape, and cell size (McBeath R. *Dev Cell*. 2004;4:483-495, Kilian KA. *Proc Natl Acad Sci USA*. 2010;107:4872-4877) affect stem cell differentiation.

Our research focused on studying the effects of cell-based biomimetic geometries on human MSC (hMSC) differentiation. We hypothesize that by restricting hMSC adhesion to mimic the shape of fully differentiated cells of the mesoderm lineage, we can direct hMSC differentiation to these specific lineages. In this work, we have restricted hMSCs to fibronectin patterns derived from the shape of a representative mature adipocyte along with a modified version of this adipocyte pattern. We used laser scanning lithography (LSL), a photothermal patterning approach, to generate arrays of these micropatterns. LSL functions by selective desorption of a protein-resistant oligo(ethylene glycol)-terminated alkanethiol self-assembled monolayer on gold, and backfilling bare gold with fibronectin, yielding micropattern arrays. hMSCs were cultured on these patterns and differentiation was examined.

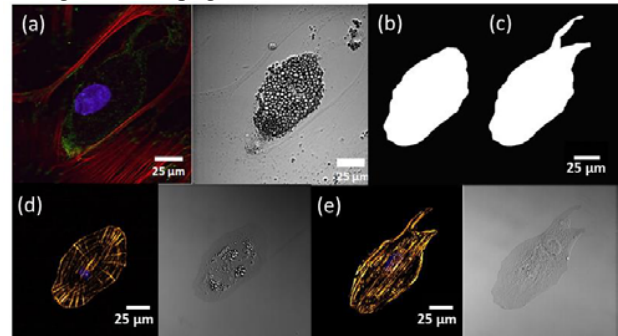
### Methods:

**Cell culture:** hMSCs and human subcutaneous preadipocytes, and all culture reagents were purchased from Lonza (Walkersville, MD). Passage 2 preadipocytes were seeded at 2500 cells/cm<sup>2</sup> on fibronectin coated cover glass for 4 weeks in differentiation media. Cells were fixed and stained for vinculin, actin, and nuclei, and imaged via confocal microscopy. Passage 4 hMSCs were cultured on protein patterns for 7 days in mixed 1:1 v/v osteogenic to adipogenic differentiation media. Cells were stained and imaged for actin, nuclei, peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ , a transcription factor associated with adipogenesis), lipid content, and alkaline phosphatase.

**Protein patterns:** Virtual masks were produced based on images of cells of interest using custom MATLAB® code. LSL was used to develop fibronectin micropatterns as previously described (Slater JH. *Adv Funct Mat*. 2011; 21:2876-2888).

**Results:** Fig. 1a shows a confocal micrograph of a representative mature adipocyte used as the basis of our studies, along with a differential interference contrast (DIC) image of this cell, where the lipid droplets are clearly visible. We generated an adipocyte-mimetic virtual mask by outlining this cell (Fig. 1b), along with a modified virtual mask (Fig. 1c) containing multiple high aspect ratio projections. These masks were used to guide LSL patterning and generate fibronectin micropattern arrays. Fig. 1d-e shows examples of hMSCs on these

patterns following 7 days in culture in a mixed 1:1 v/v osteogenic to adipogenic media.



**Figure 1. Stem Cells on Adipocyte-Based Patterns.** a) Adipocyte (left) and lipid droplets (right), b) adipocyte-mimetic pattern virtual mask, c) modified pattern virtual mask, d) hMSC on adipocyte-mimetic pattern, e) hMSC on modified pattern; (green = vinculin, red/gold = actin, blue = nucleus), Scale bar = 25  $\mu$ m.

Fig. 1d(right) shows a DIC image of an hMSC on an adipocyte-mimetic pattern with significant visible lipid droplets, as compared to the hMSC in Fig. 1e(right) on the modified pattern that lacks any visible lipid droplets. Approximately 42% of cells cultured on the adipocyte-mimetic patterns exhibited a maximum lipid droplet diameter of greater than 1  $\mu$ m, which is representative of lipid droplets found in adipocytes as compared to other eukaryotic cells (Suzuki M. *J Electron Microsc.* 2011;60:S101-S116). In contrast, approximately 18 % of hMSCs on the modified patterns showed maximum lipid droplet diameters of greater than 1  $\mu$ m. We concluded that hMSCs on adipocyte-mimetic patterns preferentially undergo adipogenesis as compared to those on modified patterns. This effect of the pattern on lipid droplet diameter was reduced in the presence of an actin inhibitor, cytochalasin D, indicating that variations in hMSC cytoskeletal tension likely influence differentiation of these cells. The increased adipogenesis of hMSCs on adipocyte-mimetic patterns as compared to modified patterns was supported by quantification of the nuclear to cytoplasmic ratio of PPAR- $\gamma$ , which was found to be 1.5 times that of the nuclear:cytoplasmic PPAR- $\gamma$  ratio for cells on modified patterns. Interestingly, hMSCs on either pattern were never found to stain positively for the early osteogenesis marker, alkaline phosphatase.

**Conclusions:** We have examined the response of hMSCs on adipocyte-mimetic patterns as compared to modified patterns, which display high aspect ratio projections. A significantly greater number of cells on the adipocyte-mimetic patterns underwent adipogenesis as compared to cells on the modified patterns. Adipogenesis was quantified based on lipid content and size, and nuclear:cytoplasmic PPAR- $\gamma$  ratios. This research has the potential to enhance *in vitro* stem cell differentiation protocols and may eventually be translated to three-dimensional tissue engineering scaffolds.