Using Biomimetic Protein Micropatterns to Guide Mesenchymal Stem Cell Differentiation

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Statement of Purpose: In order to optimize their therapeutic efficacy, it is necessary to limit differentiation of adult mesenchymal stem cells (MSCs). In attempt to mimic the natural stem cell niche, recent research has explored the effects of mechanical factors on stem cell differentiation, including substrate stiffness (Engler AJ. Cell. 2006;126:677-689), cell shape, and size (McBeath R. Dev Cell. 2004;4:483-495, Kilian KA. Proc Natl Acad Sci USA. 2010;107:4872-4877).

Cell adhesion, mediated by focal adhesion complexes, is directly linked with cytoskeletal tension, which influences intracellular signaling and cell behavior. In this work, we hypothesize that by restricting MSC adhesion to mimic adhesions formed by fully differentiated cells of the mesoderm lineage, we can direct MSCs to differentiate into these particular cell types. We first identified whether differences in adhesion characteristics exist between cells of the mesoderm lineage, including adipocytes and osteoblasts. Laser scanning lithography (LSL), a novel photothermal patterning technique, was then used to produce protein patterns mimicking adhesion characteristics of these cells. In this process, a virtual mask of a desired pattern is used to selectively desorb regions of an oligo(ethylene glycol) terminated alkanethiol self-assembled monolayer on a thin gold film using a 532 nm laser. OEG renders non-patterned regions protein resistant, while fibronectin can adsorb to the patterned areas. Differentiation of MSCs cultured on these patterns was then examined.

Methods:

Cell culture: Human subcutaneous preadipocytes, human osteoblasts, human MSCs (hMSCs), and culture media were purchased from Lonza. Preadipocytes and osteoblasts (passage 2-4) were seeded at 2500 and 1000 cells/cm^2, respectively, on fibronectin coated glass for 4-5 weeks in differentiation media. Cells were labeled for vinculin, actin, and nuclei, and imaged. MSCs (passage 3-5) were cultured on protein patterns (1 cell/pattern) for 7 days in mixed 1:1 v/v adipogenic to osteogenic differentiation media. MSCs were then stained for the presence of alkaline phosphatase and lipids.

Protein patterns: Virtual masks were produced from images of cells of interest using custom MATLAB® code. Patterns were produced via LSL using a Zeiss 5LIVE confocal microscope as previously reported (Slater JH. Adv Funct Mat. 2011; 21:2876-2888).

Results: Imaging of the focal adhesion protein, vinculin, was used to identify the presence of focal adhesion complexes in human adipocytes and osteoblasts cultured *in vitro*. Significant differences were noted between the adhesion site characteristics of these cells. There were approximately six times as many adhesions per cell area in adipocytes (0.39 ± 0.02 adhesions/μm^2) compared to osteoblasts (0.062 ± 0.03 adhesions/μm^2). Approximately 5% of the osteoblast cell area was occupied by adhesions, while 11% of the adipocyte was occupied by adhesions. These adhesions tended to be smaller in adipocytes; 93.3 ± 3.6% and 78.9 ± 5.8% of adhesions were less than 1 μm^2 in adipocytes and osteoblasts, respectively. In both cell types, 75% of the adhesions had a circularity of greater than 0.7 (where 1 is perfectly circular).

Having established that differences exist between adhesion site characteristics for these cell types, LSL was used to produce fibronectin micropatterns that recapitulate the adhesion site characteristics and shape of representative osteoblasts and adipocytes. Fig. 1a-d shows a mature adipocyte (indicated by the presence of lipids droplets), its respective cell outline and adhesion site virtual masks, and the corresponding fibronectin patterns.

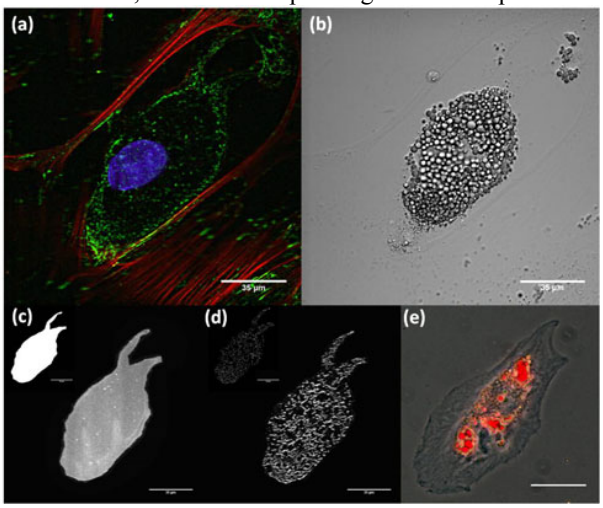

Figure 1. *Adipocyte mimetic pattern.* a) Adipocyte (green = vinculin, red = actin, blue = nucleus), b) adipocyte lipid droplets, c) outline pattern with outline mask inset, d) adhesion site pattern with adhesion site inset, e) hMSC on adipocyte outline pattern (red = lipids). Scale = 35 μm

HMSCs were cultured on these biomimetic patterns. Cells took on the pattern shape and remained confined, as shown in Fig. 1e for an adipocyte outline pattern. These cells expressed lineage-specific differentiation markers at 7 days of culture in mixed 1:1 v/v osteogenic to adipogenic differentiation media. The hMSC in Fig. 1e shows an abundance of lipid droplets (red), signifying adipogenesis on the adipocyte outline derived pattern.

Conclusions: In this work, we found that significant differences exist between the adhesion site density and size of human adipocytes and osteoblasts. LSL was used to successfully produce fibronectin patterns mimicking the adhesion site characteristics and shape of these cells. HMSCs cultured on these patterns began to express lineage-specific differentiation markers in the presence of mixed adipogenic to osteogenic soluble factors over the course of 7 days. This research has the potential to tremendously improve *in vitro* stem cell differentiation protocols and inform the design of biomaterials scaffolds for stem cell differentiation *in vivo* for a variety of regenerative medicine therapies.