Towards Degradable Protein Patterns for the Characterization of Temporal Stem Cell Behavior

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Statement of Purpose: Human mesenchymal stem cells (HMSCs) have the ability to differentiate down a variety of lineages. To increase the therapeutic potential of HMSCs, heterogeneity in differentiation must be minimized. Current studies focus on the effects of physical cues, such as substrate stiffness (Engler AJ. Cell. 2006;126:677-689), geometry (Kilian K. PNAS. 2010:107:4872-4877), and cytoskeletal organization (McBeath R. Dev Cell. 2004:6:483-495) on controlling HMSC differentiation. These effects may influence stem cell behavior in a temporal manner. Recently, it was shown that HMSCs can remember previous culture environments (Yang C. Nature Mater. 2014:6:645-652).

We hypothesize that the effects of physical constraint on stem cell differentiation are time-dependent. In order to investigate this hypothesis, we have developed degradable substrates for stem cell confinement in two-dimensions using alkanethiol self-assembled monolayers (SAMs) formed on thin, nanometer-scale metal films. Alkanethiols can be conjugated to a variety of functional groups, which affect properties such as surface wettability. Our tunable surfaces undergo controlled degradation based on material properties without the need for any external stimuli.

SAM stability is a function of metal to sulfur bond strength, metal thickness (Petrovykh D. Langmuir. 2006:22:2578-2587), alkanethiol carbon chain length, and end group functionality. Alkanethiols form a semi-covalent bond with the metal. As the metal oxidizes over time, the SAM degrades. By varying the thickness of the metal layer and metal type, we have developed degradable alkanethiol SAMs that are stable for varying lengths of time. We are now examining HMSC behavior on our tunable materials.

Methods:

Cell Culture: HMSCs (Lonza) were cultured in mixed media containing 1:1 (v/v) osteogenic:adipogenic factors at 37ºC and 5% carbon dioxide.

Migration Studies: A thin layer of metal, gold (Au) or platinum (Pt), was electron-beam evaporated onto coverglass, and a cross-shaped region of the metal was etched away, as seen in Figure 1. Hydrophilic 2-{2-[2-(2-{2-[2-(1-mercaptoundec-11-yloxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethanol (OEG6) alkanethiol SAMs were deposited on the remaining metal. Human plasma fibronectin was then adsorbed to the samples. HMSCs were first treated with mitomycin C to inhibit proliferation and then seeded onto the substrates at 8800 cells/cm^2. Fixed samples were stained with DAPI for nuclei and AlexaFluor® 532-phalloidin for F-actin and imaged on a Nikon TiE inverted fluorescence microscope.

Results: Proteins and, therefore, cells adhere more readily to hydrophobic surfaces. In this study, the hydrophilic OEG6 is protein resistant. The fibronectin adsorbs to the regions of bare glass exposed during substrate fabrication. Cells were found to initially adhere to the fibronectin-coated glass regions only (Figure 1A, day 1). As the hydrophilic SAM degrades, the cells can migrate beyond the initial confined regions, allowing us to investigate the substrate stability and cell behavior over time.

Cellular migration over time on 8 nm Au is demonstrated in Figure 1A. Figure 1C quantifies cellular migration on the 8 nm Au, demonstrating that degradation of the SAM begins at day 7 with approximately 27% cellular migration away from the initial confinement of the scratch.

To investigate the effects of metal thickness on SAM stability, we examined HMSCs on 10 nm Au. Figure 1B demonstrates cellular migration over time on 10 nm Au. Figure 1C also quantifies cellular migration on the 10 nm Au, demonstrating that degradation of the SAM begins at day 5 with approximately 29% of the cells migrating off of the initial glass confinement.

We also examined the effect of metal type on SAM stability by observing HMSCs on 12 nm Pt. HMSCs migrated off of the exposed glass region in as little as 3 hours after seeding the cells, as seen in Figure 1D.

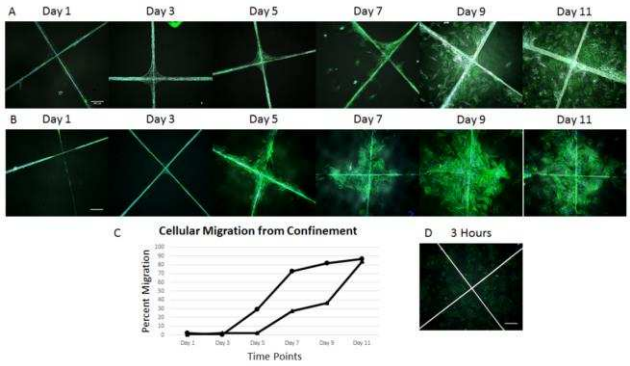

Figure 1: A) HMSC migration onto initially non-adhesive regions over time on 8 nm Au. C) HMSC migration onto initially non-adhesive region over time on 10 nm Au. C) Percent cell migration off etched region for 8 nm (triangles) and 10 nm Au (circles). D) HMSCs on 12 nm Pt at 3 hours. Green is F-actin, blue are nuclei. Scale is 200 μm.

Conclusions: In this study, we have demonstrated that alkanethiol SAMs may be used to fabricate degradable surfaces that allow for the temporal confinement of cells. We have shown that by altering metal thickness and type, we can vary the duration of stability of these surfaces. We are currently examining the differentiation behavior of HMSCs on these SAM surfaces and developing controlled geometric features for similar studies of temporal cell behavior in the future. This research could impact the field of stem cell research and increase the therapeutic potential of HMSCs.