Innovative Injury versus Non-Injury Migration Assays

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Statement of Purpose: Cellular migration is important when considering many tissue engineering strategies. It plays a vital role in vascular remodeling, wound healing, and tissue repair. Quantification of cellular migration can provide important information about cellular activity as cells interact with each other and surfaces. Common assays used for quantifying migration injure the cells and could potentially influence migration. In this study, two simple, scalable non-injury migration assays were developed to study the effects of different ECM surfaces on cellular migration without the effects of injury. The results were examined and compared to a commonly-used Scrape Wound assay.

Methods: Non-injury assays looked at the out-migration of smooth muscle cells (SMC) and human umbilical vein endothelial cells (HUVEC) seeded on the inside of glass cylinders (cylinder assay) and in-migration of SMC and HUVEC seeded on the outside of PDMS stamps (PDMS lift-off assay). The injury scrape wound assay involved SMC and HUVEC seeded on a clean surface and then scraped with a sterilized cottonwood stick. All cells were seeded for 4 hours at 37°C on either Collagen I, Collagen IV, Fibronectin, Laminin, Gelatin, or no matrix surface. Migration was measured by tracing the borderline of migratory cells using ImageJ software. Percentage of migrated cells was calculated from a baseline value of zero hour migration.

Results: Optimal adhesion and migration rate for SMCs and HUVECs were found on collagen-based proteins (Collagen I, Collagen IV, Gelatin). For Collagen IV, the percent of migration over time for HUVECs and SMCs under the scrape-wound injury assay is 0.30 and 0.35, respectively (Figure 1 and 2). For HUVECs on Collagen IV, the rate of injury migration is about 20% higher than non-injury migration assays; while SMCs migrate 20% faster than PDMS stamp and 34% faster than hollow cylinder. Injuring the cells releases more cell-signals for migration and proliferation to facilitate the healing process.

Conclusions: The injury model proved to have greater cell migration on both SMC and HUVEC. Injuring the cells creates more cell-signaling for the cells to proliferate and migrate. These paracrine/endocrine effects are present in the Scrape Wound assay and absent in the Cylinder and PDMS lift-off assays. The developed assays provide a clean process in which cells migrate without the activation of injury mechanism and can be useful for emerging anti-atherosclerotic pharmacologic agents.

Figure 1

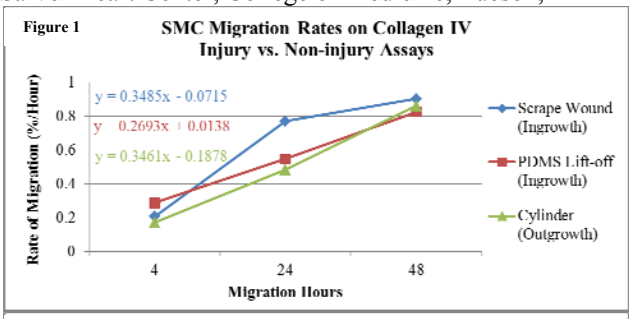
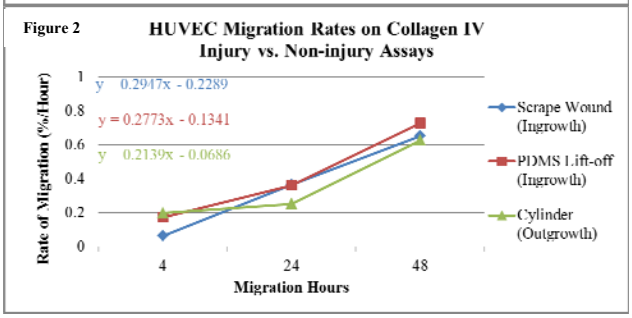


Figure 2



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