Filling the Gap: The Relative Role of Proliferation versus Migration in the Response to Injury of Vascular Smooth Muscle Cells

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Statement of Purpose: Wound healing is a universal biological response to injury. In the world of vascular biology, the response of smooth muscle cells (SMCs) to injury is of great importance, not only for understanding basic mechanisms but also in tackling the clinical development of post-angioplasty restenosis and post-surgical neointimal thickening. For context, up to 60% of balloon angioplasty cases result in restenosis [1-3]. Our lab has been studying mechanisms of response of vascular SMCs to injury [4]. In this response SMCs over time typically fill a "gap" or wound associated with the injury. It has long been felt that both migration and proliferation of cells contribute to this gap-filling process. However, the relative percentage of proliferation versus migration as a response to injury remains unknown. In this study, we initiated our investigation in this area by looking at global or overall population proliferation vs migration using three well-described assays [5], several of which have been developed by our lab. We hypothesize that proliferation is only a partial component of net cellular mechanisms in "closing the gap" associated with SMC wounding in culture.

Methods:
Two non-injury migration assays: 1) hollow cylinder for out-migration and 2) polydimethylsiloxane (PDMS) lift-off stamp for in-migration, were used to quantify SMC migration in comparison to a scrape-wound injury model. Cells were seeded for 4 hours at 37°C and 5% CO_2 for each assay. Non-injury barriers were then removed or the monolayer was scraped, establishing a "wound" at the 0 hour time point. Cells were then allowed to migrate and proliferate for 4, 24, and 48 hours at 37°C and 5% CO_2. Migration and proliferation (CyQUANT Proliferation Assay™) were quantified by the total cell area and total number of cells, respectively. All data was normalized to the 0 hour measurements to yield a percent change in migration or proliferation.

Results:
All assays exhibited a steady increase in migration over the 4, 24, and 48 hour period. However, proliferation did not exhibit a steady increase over time for every assay. Only the PDMS lift-off assay had a constant increasing trend of proliferation with a 7.7%, 32.6%, and 51.4% increase in total cell number at the 4, 24, and 48 hour time point, respectively. The cylinder assay and scrape wound assays had a much higher initial increase in cell number at the 4 hour time point with a 32.0% and 30.0% increase, respectively. After longer time points, the scrape wound assay does not change significantly in cell number and peaks at 24 hours with a 32.0% increase. This is in contrast to the cylinder assay which also peaks at 24 hours but with a 81.1% increase.

Conclusions: Proliferation is a significant, but only partial (up to 51%) component in filling the gap created in both wounding and non-wounding (release of contact inhibition assays) of SMC monolayers. To dissect this further our next step is to explore the spatial localization of the proliferation geographically in relation to the wound leading edge. Understanding the relative role and distribution of migration and proliferation in vascular injury will ultimately allow the designing of novel therapeutic strategies which address underlying mechanisms involved

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