Paired computational and experimental approaches probing stem cell adaptation to volume-and shape changing stresses

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INTRODUCTION: Mechanical cues play a ubiquitous role in biological processes, underpinning the emergence of structure-function relationships in development and healing. *Mechanomics* describes stem cell (SC) adaptation to the mechanical environment, regulates SC cytoskeletal organization, and dictates lineage commitment, guiding the emergence of structure and function in developing tissues. To probe the mechanome of live SCs there is a growing need for robust tools to deliver highly controlled mechanical cues. Here we present a novel tool pairing computational and experimental delivery of volume- and shape-changing stresses, by increasing seeding density and introducing laminar flow, respectively, while perturbing the microtubule cytoskeleton with paclitaxel (PAX).

METHODS: We delivered controlled volume- and shape-changing stresses by increasing seeding density[1,2] and laminar flow (0.2 dyn/cm^2)[3–5], respectively, shown previously to induce local compression and shear stress. Cells of the C3H/10T/1/2 murine embryonic SC line (passage <15) were seeded at low density (LD, 5000 cells/cm^2), high density (HD, 15,000 cells/cm^2) and very high density (VHD, 45,000 cells/cm^2), and treated with distinct concentrations of Paclitaxel (PAX, 1-100nM) to control cytoskeletal dynamics. Live imaging was performed to visualize and measure SC adaptation within 60 minutes of flow exposure. RT-PCR was performed using RNA extracted from cells of all density and PAX treatment groups, to probe mesenchymal condensation genes and other differentiation markers.

RESULTS: PAX treatment was associated with cell volume increase and flatter shape, in a time- and concentration-dependent manner, concomitant to increase in F-actin alignment, F-actin and microtubule concentration per cell, and cell stiffness. Higher seeding density exerted a greater effect than PAX exposure in modulating cell volume increase, as well as F-actin and microtubule concentration increases. Paired computational and experimental delivery of flow revealed how SC structure, as well as changes in shape due to PAX treatment and seeding density, profoundly influence flow fields around and along SCs' apical to basal height. SCs' immediate responses to flow exposure included optimization of shape and height; SC surfaces moved closer to the substrate over time, effectively reducing drag. Of note, control cells adapted more dynamically than PAX treated cells in regulating their flow-induced displacements (Fig. A). Actin remodeled by repositioning of filopodia, while microtubules became more saturated under flow, and thick bundles of microtubules in PAX-treated cells moved collectively to protect the nuclei relative to flow direction (Fig. B). PAX treatment was associated consistently with upregulation in actin and tubulin mRNA; this was enhanced by flow exposure yet diminished with increasing seeding density (Fig. C). Flow and high seeding density upregulated msx2, col1a1, and col2a1, the respective markers for pre, peri- and post mesenchymal condensation, in a complementary manner. In contrast, expression of *acan*, *sp7*, and *pecam-1*, the respective chondrogenic, osteogenic and angiogenic markers, was abrogated either by flow or increasing seeding density (Fig. D,E,F).

DISCUSSION: The interplay of mechanical and chemical cues, introduced in this study via increasing seeding density, exposure to flow and a range of PAX concentrations, were shown to influence SC differentiation profiles and show the potential for tuning of mechanical cues to achieve target lineage commitment. For example, as single cells evolve to develop multicellular constructs, the local compression could be orchestrated with flow to upregulate mesenchymal condensation markers, or flow could be used to abrogate the upregulation of skeletal differentiation that was observed at high seeding density with PAX treatment.

SIGNIFICANCE/CLINICAL RELEVANCE: The mechanomics engineering tools used in this study present a novel means both to map the mechanome of live SCs as well as to elucidate the role of mechanics in lineage commitment. Next generation materials and medical devices may harness mechanomics to guide cell fate and tissue genesis in context of regenerative medicine.

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IMAGES AND TABLES: