INTRODUCTION: A stem cell’s shape and fate is intrinsically linked to its form and function [1]. Stem cells are mechanosensitive, exhibiting an exquisite capacity to sense and respond to their local mechanical environment [1-5]. Embryonic stem cells change baseline gene activity in response to seeding conditions, as well as short duration (15-30 minutes), small magnitude (0.1 dyn/cm²) shear stresses [4,5], further emphasizing the importance of mechanical signals in cell differentiation. Stem cells use this capacity as they assemble tissue templates and specialize those templates into functional tissues. [1]

Our working hypothesis is that the structure of a stem cell, modulated to a large degree by its cytoskeletal arrangement, is dynamically determined by the cell’s local mechanical milieu. In a dynamic environment, anisotropic structural adaptations emerge in cellular architectures and cellular differentiation and tissue formation ensue in due course. The goal of this study is to elucidate dynamic structural remodeling (mechanical adaptation) of embryonic stem cells. Our approach is to characterize spatial and temporal changes in the actin and tubulin components of the embryonic mesenchymal stem cell cytoskeleton in response to flow induced shear stress and different seeding densities. Low magnitude, steady fluid flow mimics the environment of embryonic stem cells prior to the first beating of the embryonic heart or the first twitch of skeletal muscle [6]; varying seeding densities emulate different developmental stages, particularly in context of the mesenchymal condensation, which marks the first stage of skeletal development [4,5].

METHODS: Experiments were carried out using the C3H/10T1/2 murine, embryonic mesenchymal stem cell line (ATCC, Manassas, VA). All cells were derived from passage 5/6 and cultured in a Basal Medium Eagle supplemented with 10% FBS, 1% L-glutamine, and 1% Pen/Strep at 37°C and 5% CO₂ in a humidified incubator. Cells were seeded on 25mm plain glass coverslips that were treated with radio frequency plasma discharge (Harrick Plasma, Bhaca, NY) for 2 min. All samples were prepared 24 hours prior to the flow experiments using an optimized protocol for live labeling of the actin and tubulin components of the cytoskeleton (Cellular Lights™ Actin and Tubulin GFP, Invitrogen, Carlsbad, CA). Samples were subjected to 0.5 and 1 dyn/cm² shear stress for 90 minutes, using a parallel plate laminar flow chamber designed to impart highly controlled shear stresses [6,7]. Confocal fluorescent image stacks (300 µm × 300 µm images, taken at 0.2 µm increments) of live cells were taken every 30 minutes. Cells not exposed to flow (static) served as controls. Images of cells were analyzed using Volocity (Improvision Inc., Waltham, MA) and Matlab (Mathworks, Natick, MA) to quantify actin and tubulin distribution in space and time (Fig. 1.2). Statistical analysis was done using JMP 8 (SAS Institute Inc., Cary, NC). Significance was determined using matched pair analysis where | prob > |z| | < 0.05 (Wilcoxon signed rank test). Correlation was determined using nonparametric analysis where p < 0.05 (Spearman rank correlation).

RESULTS: Seeding density impacts both size and shape of the cells, as well as cytoskeletal organization. A low density seeding of cells at 5,000 cells/cm² resulted in larger cells with more defined cytoskeletal structures. A high density seeding of cells at 35,000 cells/cm² resulted in smaller cells with a more compact and less defined cytoskeletal structures.

The spatiotemporal distribution of actin and tubulin were measured with respect to the direction of flow as well as the base of the cell. High density samples show more robust adaptation in response to 1.0 dyn/cm² shear stress than cells exposed to 0.5 dyn/cm². Remarkably, the total amount of actin in the cytoskeleton increased after 30 and 60 minutes of high flow. Furthermore, the thickness of the actin cytoskeleton increased after 60 and 90 minutes of flow. In high density samples, the structural remodeling of the actin structures were not significantly different when comparing low flow and non-flowed control samples. In contrast, samples cultured at low density appear to show a more robust adaptive response when subjected to low flow compared to high flow conditions (data not shown).

DISCUSSION: Based on these studies, the spatial distribution of the cytoskeleton is modulated through the cell’s local mechanical milieu. Stem cells exhibit a significant increase in actin upon exposure to minute mechanical signals. The amplification of actin at the base of the cell suggests an anchoring and stiffening of the cell in response to high flow, revealing a more organized and directed response to shear stress that previously known. Furthermore, the space surrounding cells (a direct manifestation of cell density) affects not only on cell shape and size but also cellular structure and mechanosensitivity. High resolution flow imaging studies show an increase in flow rate (and hence shear stress imparted at interfaces) in spaces between cells, much like at the narrowings in a stream [8]. Hence, stem cells exhibit an innate capacity to sense their immediate environment and strengthen their structural systems to protect themselves. Novel spatiotemporal imaging methods and computational models enable us to probe nascent anisotropic mechanical properties and mechanical at the length scale of a single cell. Ultimately we aim to decipher how mechanical signals modulate the adaptation of cells to their environment via enforcement of cell shape and structure. This will further bring to light the mechanisms behind stem cell fate commitment, both during prenatal development, wound healing and regeneration, as well as de novo engineering of tissues.
