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MECH 498A

Cell migration is a fundamental physiological process in normal physiology and pathology. The cell nucleus is emerging as a key player in this process. Being the largest and stiffest organelle, in eukaryotic cells, the nucleus acts as a limiting factor during cell migration. The role of nuclear mechanics in cell migration is therefore a topic of recent interest to both the basic science and clinical research communities. The objective of the proposed work is to study how nuclear mechanical properties affect collective cell migration using a model system of the scratch wound assay. Quantitative understanding of collective cell migration will be obtained using live time lapse imaging of cells with modified nuclear stiffness and chromatin remodeling.

Specific Aim #1: Establish a scratch wound assay of cell migration using NIH-3T3 cells. NIH-3T3 mouse embryonic fibroblast cells will be used in this study because they are an excellent model system for this assay and easy to culture. Establishment of a scratch wound assay will involve culturing of the cells in a confluent manner and applying the scratch in a consistent manner. Migration parameters such as speed, persistence, and closure potential will then be quantified using image processing in Fiji/ImageJ. Determining these image processing methods will also be needed for Aim 2. The assay technique will then be validated using one or two migration blockers such as cyto-D to inhibit actin or Y27 to inhibit actomyosin contraction.

Specific Aim #2: Observe how changes to nuclear mechanics affect migration parameters. After establishing the control, drugs will be applied to the NIH-3T3 cells modifying their nuclear stiffness and chromatin remodeling. These cells will then undergo the scratch wound assay established in Aim 1. Subsequently, migration parameters will be quantified using image processing and will be compared to the results found in the control.

Specific Aim #3: Perform live imaging of the cytoskeleton and nucleus at high resolution to quantify a strain map in the cell monolayer. The scratch wound assay will be repeated for both the control and modified cells with visualization of the cell cytoskeleton and nucleus using live staining of actin and DNA, respectively. Live imaging at a high resolution will then be performed on these cells during migration. Using image processing, a strain map will be generated at the cell monolayer providing detailed mechanical insight into the cell migration process.

Determining the effects of nuclear mechanical properties on cell migration, and how these effects pertain to the cell monolayer, will help increase our understanding of this fundamental physiological process. If the aims of this project are successfully met, it would help lay groundwork for further studies in understanding the synergistic role of nuclear mechanics with cell crowding effects and differing substrate mechanics. The knowledge will provide as the baseline information to be applied in tissue engineering applications.