Effect of Different Extracellular Matrix Proteins on Collective Cell Migration

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Abstract:

Studying collective cell migration is essential for understanding physiological processes, such as wound healing and cancer metastasis [1]. Since the extracellular matrix (ECM) interacts with cells, through contact guidance and haptotaxis, it is essential to understand how the different ECM proteins affect cell migration patterns [2]. Previous research done in Nakanishi-san's group (by Dr. Shimaa A. Abdellatef) demonstrated how single cell migration patterns vary as the ECM proteins differ, but there is a lack of research on the effect of the ECM on collective cell migration. Unfortunately, the ECM is disrupted during conventional methods for studying collective cell migration (e.g., scratch wound assays). In this study, a more advanced technique using photoswitchable passivated surfaces was tested and proved to be suitable in observing the cell migration on different ECM proteins. This method achieved region specific ECM adsorption with a significant difference between the specific and nonspecific adsorption. Cells were seeded and attached to the ECM and then were observed as they migrated. The cell migration results suggest that the ECM type is critical to collective cell migration, as the cell clusters exhibit different migration patterns with varying ECM proteins.

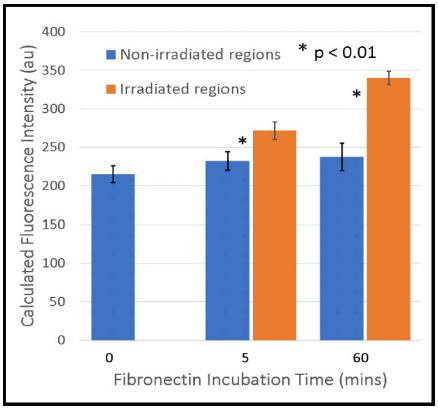

Figure 1: Immunofluorescence results of radiated and nonirradiated regions for samples with different protein incubation times.

Summary of Research:

Photoswitchable passivated glass surfaces have been used to achieve region specific cell adsorption to observe cell migration with no harm to the cells [3]. In this method, the glass has a coating that detaches from the surface upon exposure to ultraviolet (UV) light. Region specific cell adsorption is possible since cells can only attach the irradiated regions. It was hypothesized that this technique could be used to attain region specific ECM adsorption.

Testing Protein Adsorption to Photoswitchable Passivated Glass Surfaces. To take advantage of the photoswitchable passivated glass for this study, the ECM must adsorb onto the glass surface with significant difference between the specific and nonspecific adsorption. To assess the ECM adsorption, the passivated surface was irradiated with circle patterns and exposed to fibronectin for varying incubation times. Ideally, the fibronectin would only adsorb onto the irradiated regions. The samples were then exposed to a primary antibody for fibronectin attached to a fluorophore so the samples could be observed under immunofluorescence.

The immunofluorescence results demonstrated significant difference in calculated fluorescence intensity between the irradiated and non-irradiated regions for both the 5-minute and 60-minute samples (see Figure 1). The irradiated regions of the 60-minute sample also

exhibited a greater calculated fluorescence intensity compared to that of the 5-minute sample. These results prove that fibronectin adsorbs to the surface and that the higher incubation time resulted in a higher density of fibronectin. Since fibronectin was demonstrated to adsorb to the surface with significant difference between specific and non-specific adsorption, this technique using the photoswitchable passivated glass surface was suitable to observe the effect of different ECM proteins on collective cell migration.

Observing Cell Migration. Once the samples had region specific ECM adsorption, cells were cultured onto the samples (see Figure 2). The region outside the cell cluster was then irradiated to remove the coating and more ECM was added. During migration, pictures of the cell clusters were taken every 30 minutes for three hours. The area of the cell clusters was measured every 30 minutes (see Figure 3).

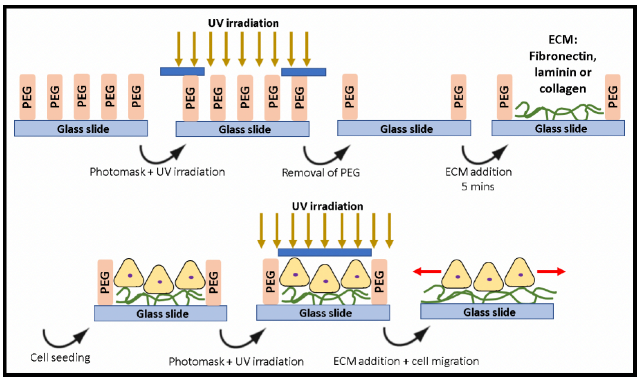

Figure 2: Method using the photoswitchable passivated glass to attain region specific ECM adsorption and observe cell migration.

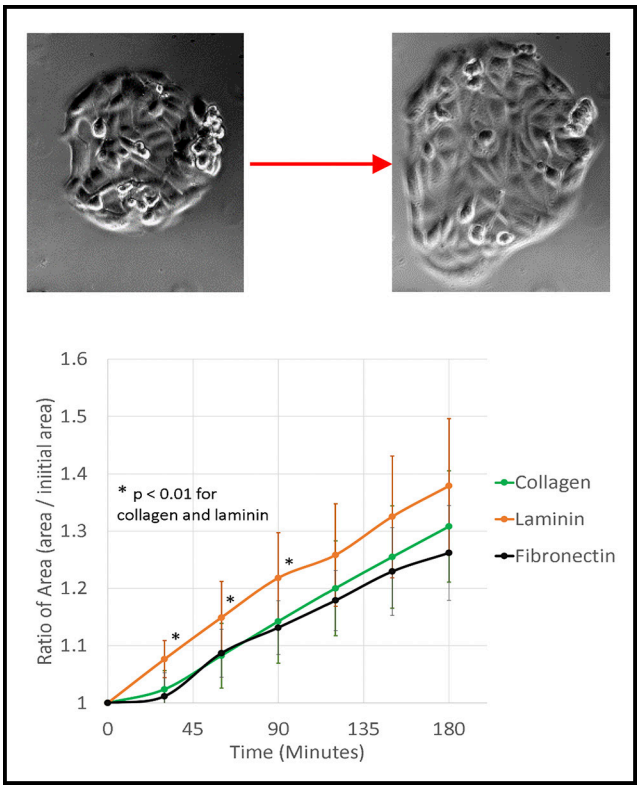

Figure 3: Ratio of area expansion of cell clusters of cells migrating on different ECM proteins with images of the cell cluster on a laminin coated surface at 0 and 180 minutes.

The cell migration was observed with different ECM proteins: fibronectin, collagen, and laminin. Based on Figure 3, there is a significant difference in area expansion between the cells migrating on collagen coated surfaces compared to that on laminin for the first 90 minutes. This difference in migration suggests that ECM proteins are critical to collective cell migration.

Conclusions and Future Steps:

Understanding how ECM can help or hinder collective cell migration would allow for the manipulation of physiological process such as speeding up wound healing or hindering cancer metastasis. Since conventional techniques for observing collective cell migration disrupt the ECM, a more advanced technique was necessary. This study proved a method using photoswitchable passivated surfaces is suitable to observe collective cell migration on different ECM proteins. Fibronectin, an ECM protein, was demonstrated to adsorb onto the surface in specific patterns with a significant difference between specific and nonspecific adsorption. A greater protein incubation time resulted in a higher density of protein adsorption. After achieving region specific ECM adsorption, cells were seeded and attached to the ECM. The collective cell migration results suggest that the ECM type is critical to migration.

The next steps of this study include confirming that other ECM proteins, like collagen and laminin, also adsorb onto the glass surface through similar immunofluorescence experiments. Further, cell migration patterns are significantly influenced by the cell culturing (i.e., density) before the cells are seeded onto the samples. Therefore, for the fairest comparisons between cell migrations on different ECM proteins, the seeded cells on each sample should come from the same cell cultures. It is important to note that the results in Figure 3 reflect multiple cell migration experiments where the cells were from different cell cultures, which led to large deviation.

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