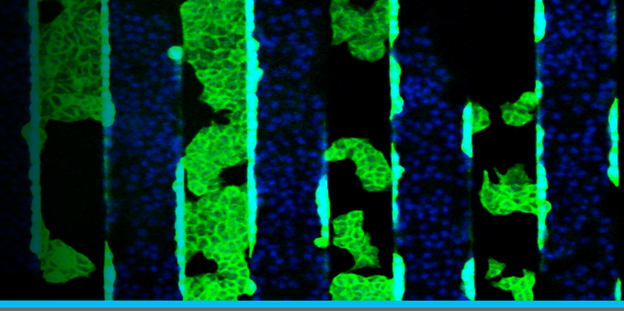
Understanding how cells sense their microenvironment



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Introduction

Cells *in vivo* are strongly influenced by biochemical signals and physical cues, such as different forces, mechanical properties and topographies which they encounter in their respective microenvironments [1-4]. These cues have been shown to regulate differentiation, migration and proliferation of cells [3,5]. A recent study determined that the micro-topography of the cellular microenvironment can influence cell migration and organization in three-dimensions [5]. When two different cell types were allowed to grow in micro-fabricated grooves, it was shown that fibroblasts display a clear preference for proliferating along groove ridges, whereas epithelial cells prefer to proliferate in the grooves [5]. In an attempt to further examine cellular responses to physical cues, this study aimed to alter the migration patterns of epithelial cells by manipulating the intercellular connections. Cadherins are transmembrane proteins that play important roles in cell-cell adhesions [6]. It was hypothesized that the organization of cells with blocked cadherin connections would differ from cultures of cells with expressed cadherins [7]. The goal of this project was to optimize the cadherin blocking process in order to carry out future experiments that will contribute to new mechanistic knowledge pertaining to how a cell is able to sense its physical environment.

Methodology

MDCK cell lines were grown in DMEM media with 10% FBS and 1%P/S, and incubated at 37 °C

↓

250,000 cells were incubated in serumless DMEM media with 1/100 concentration of primary e-cadherin antibody for 30 min

↓

Cells were allowed to grow in DMEM media for 48 hours

↓

Cells were fixed with PFA, and then stained for dapi and cadherin

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Same process was carried out for a control sample of MDCK cells that were not incubated with primary e-cadherin antibody

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Both samples were imaged and analyzed with confocal microscopy

Results

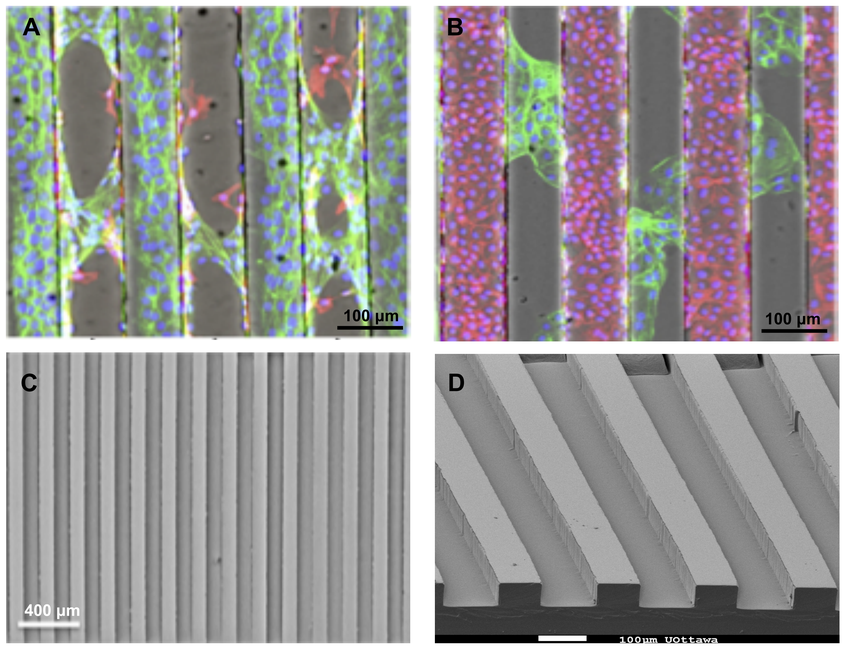

Figure 1. NIH3T3 cells (A) proliferate along groove ridges after 48h of growth [5]. MDCK cells (B) proliferate within grooves after 48h of growth [5]. (C) A top-down SEM image of the PDMS substrate reveals the structure of a typical microtopography with 100 mm grooves and ridges. (D) A 3D view of the grooves.

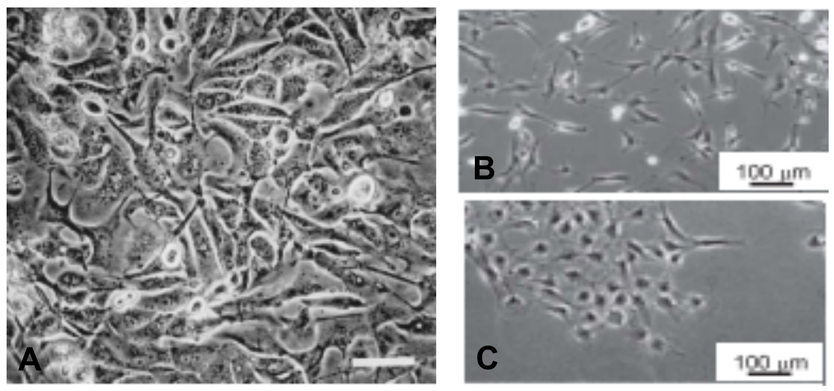

Figure 2. MDCK cells (A) organization after being grown with e-cadherin antibodies for 48 h (scale bar = 50 µm) [7]. NIH3T3 cell organization before (B) and after (C) e-cadherin over-expression [8].

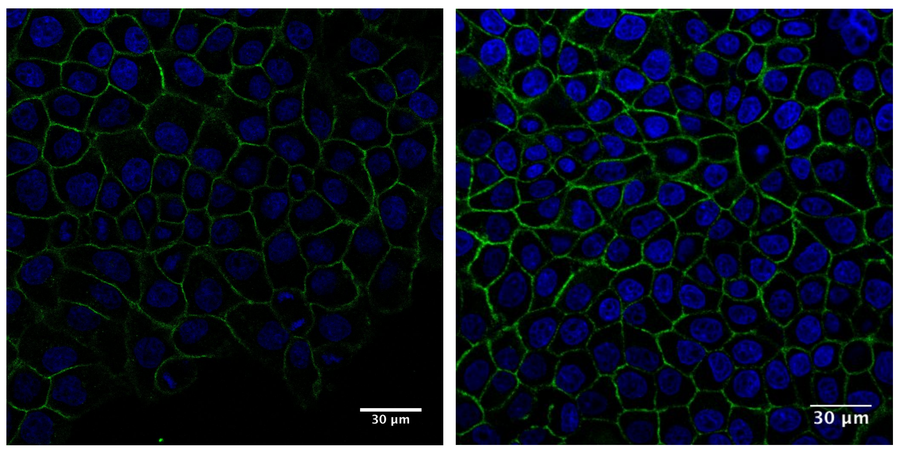

Figure 3. Confocal microscopy images of MDCK cells after being grown with (A) and without (B) e-cadherin antibody for 48 h. Nuclei are shown in blue and cadherins are shown in green.

Conclusion

The results show that allowing 250,000 MDCK cells to be grown with e-cadherin antibody for 48 hours does not have an effect on the organization of MDCK cells. No separation between MDCK cells was seen after attempting to block the cadherin connections between cells. According to past literature, this phenomena should occur. Therefore, it is presumed that with different conditions, the MDCK cells will organize themselves as in Figure 2A.

Future Directions

- → Further optimize e-cadherin blocking protocol with MDCK on flat surface. Changes will include lowering initial MDCK cell count, incubating the cells with e-cadherin antibody in different blocking media, and growing the cells in serumless DMEM media.
- → Optimize over-expression of cadherins in NIH3T3 on flat-surface to resemble growth of MDCK such as in Figure 2C [8].
- → Attempt to reverse results of previous study [5] by e-cadherin blocking MDCK cells on grooves and by over-expressing cadherins in NIH3T3 cells on grooves.

References

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Acknowledgements

Special thanks are extended to Dr. Andrew E. Pelling for his guidance and patience, to Sebastian Hadjiantoniou for his mentorship, and to all other members of the Pelling Lab. Thanks also to the NSERC Discovery Grant for funding the project and to UROP for this great opportunity.

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