Long-Term Effect of Confinement on Cell Speed and Bioenergetics of Migratory Breast Cancer Cells

Addison White[1], Jenna Mosier[1], Cynthia Reinhart-King[1]
[1]Department of Biomedical Engineering, Vanderbilt University

INTRODUCTION

- Metastasis accounts for 90% of cancer-related deaths.
- Metastatic breast cancer cells invade the extracellular matrix (ECM) from the primary tumor.
- Physical properties of the primary tumor microenvironment have been shown to influence cell migration and metabolic pathways.
- The long-term effects of cell confinement ("mechanical/metabolic memory") are largely unknown.

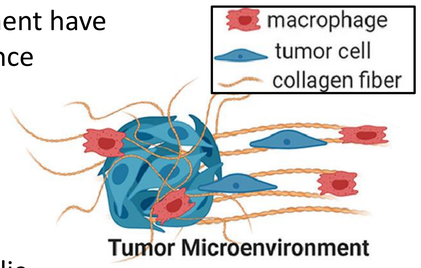

Figure 1: Invasive tumor cells interact with collagen fibers of ECM[1].

METHODOLOGY

- Used PDMS stamps to create collagen microtracks mimicking the ECM (Fig. 2)
- Each microtrack contained regions of varying widths to model changes in cell confinement
- Microtracks seeded with MDA-MB-231 breast cancer cells at ~100,000 cells/mL

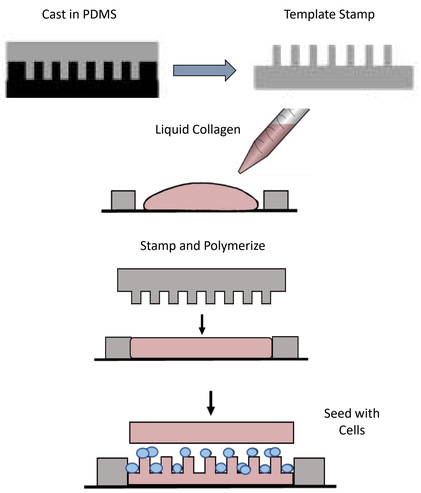

Figure 2: Microtrack molding process[2].

ENERGY UTILIZATION

- PercevalHR probe allowed energy utilization to be quantified as ATP:ADP ratios as the cells migrated from confined to unconfined regions of the microtracks
- Fluorescence measured under LSM800 confocal microscope and analyzed via ImageJ

RESULTS

Cell Behavior is Altered by Confinement

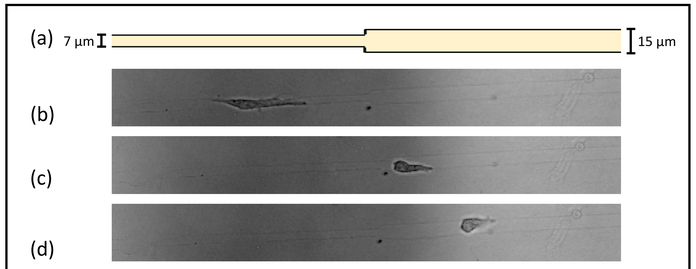

Figure 3: (a) Geometry of microtracks, (b-d) Confocal images of MDA-MB-231 cell migrating within (b) full confinement, (c) the transition point between full and partial confinement, and (d) partial confinement.

- Speed ramps up as cell travels in full confinement, then begins to decrease through the unconfined region

Cell Velocity Dependent on Distance in Confinement

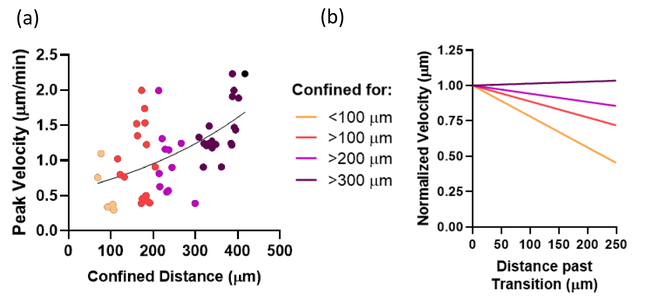

Figure 4: (a) Maximum velocity reached by cells confined for varying distances, (b) Average velocities of cells after leaving confinement.

- Longer confinement periods correlated with greater peak velocities (Fig. 4, a)
- Differences may be due to ability to adhere to microtrack when fully confined
- ***Mechanical memory:*** Cells confined for longer distances were able to maintain high velocities for longer after leaving confinement (Fig. 4, b)

RESULTS, cont.

Metabolic Memory Dependent on Distance in Confinement

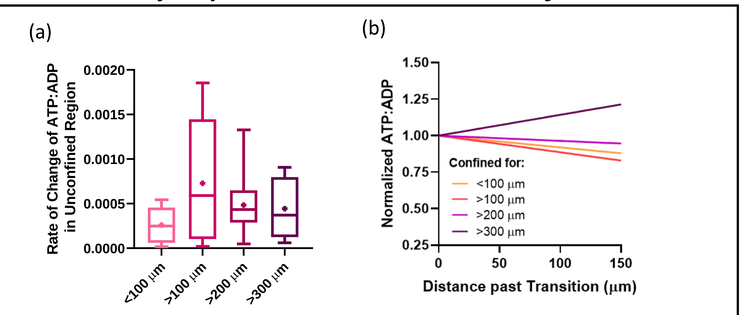

Figure 5: (a) Absolute values of average rates of change of ATP:ADP ratios after leaving confinement for cells of varying confined distances, (b) ATP:ADP ratios of cells after transitioning to partial confinement.

- Longer confinement distances correlated with slower rates of change in energy utilization after leaving confinement (Fig. 5, a)
- ***Metabolic memory:*** Cells which spent longer distances in confinement maintained metabolic trends for longer (Fig. 5, b)

CONCLUSIONS

- Both the velocity and energy utilization levels of migratory breast cancer cells increase under conditions of full confinement.
- After leaving confinement, migratory cells appear to maintain a temporary mechanical and metabolic memory which is dependent on the distance traveled in confinement.

Future Directions

- To what extent does ATP machinery (primarily mitochondria) relocalize towards the leading edge?
- Applications in therapeutic targets in breast cancer treatment

ACKNOWLEDGEMENTS & REFERENCES

The authors of this poster would like to thank funding sources at both the NIH (GM131178) and NSF-GRFP (DGE-1937963) to J.A.M., as well as the VINSE REU grant to A.E.W. (NSF-DMR 1852157). Special thanks to the Reinhart-King lab, Sarah Ross, and Alisha McCord for their support, as well as VINSE and cleanroom staff for their guidance in the microfabrication portion of this project.

[1] Szulczewski et al., *Acta Biometer.*, 2021.
[2] Mosier, Rahman-Zaman, et al., *Biophys. J.*, 2019.