Investigating Metabolic Regulation of Cancer Stem-Like Cells in the Perivascular Niche

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Primary Source of Research Funding: 2021 Cornell NanoScale Science & Technology Facility Research Experiences for Undergraduates (CNF REU) Program via National Science Foundation under Grant No. NNCI-2025233

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Primary CNF Tools Used: ABM contact aligner, Heidelberg mask writer - DWL2000, Hamatech 9000

Abstract:

A population of cancer cells known as cancer stem-like cells, or CSCs, promote mortality in breast cancer by driving metastasis and relapse. These cancer cells interact with the extracellular matrix, other cell types, secreted factors and the perivascular niche, the region directly next to blood vessels. One of the major components of the perivascular niche are the endothelial cells, which secrete factors that regulate stemness properties. The perivascular niche also contains a unique metabolic microenvironment, which affects the metabolic behavior of CSCs. In this study, we created a microfluidic device that features a large central reservoir for collagen, four media reservoirs and two channels for seeding breast cancer and endothelial cells. These microfluidic devices can be used to help better understand the tumor microenvironment and determine how targeting the CSC population could help to prevent or treat late stage breast cancer.

Summary of Research:

Introduction. Metastatic breast cancer presents a significant and unmet clinical need. Breast cancer is the leading type of cancer among women with one in eight women developing breast cancer at some point during their lives [1]. Cancer stem-like cells (CSCs) are known to express specific stem cell markers and demonstrate other properties, such as therapeutic resistance and self-renewal, which allows these cells to evade therapy and repopulate the tumor resulting in relapse [2]. However, the factors that contribute to the emergence of CSCs in tumors are still not well understood.

During metastasis, CSCs move through circulation, spread to distant tissues and interact with the perivascular niche, the region directly next to blood vessels. One of the major components of the perivascular niche are the endothelial cells, which secrete factors that regulate stemness properties such as self-renewal and invasion. The perivascular niche also contains a unique metabolic microenvironment, which affects the metabolic behavior of CSCs. There is currently little research on how endothelial cells together with metabolic diffusion affect the metabolism of tumor cells.

To this end, we plan to use a microfluidic cell culture system (Figure 1). Microfluidics will allow us to precisely control metabolic gradients and spatial organization of cells to uncover how perivascular niche factors affect CSC metabolism and subsequent stemness.

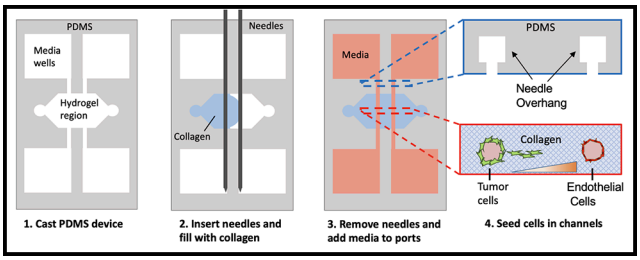

Figure 1: The microfluidic device fabrication process.

Design and Fabrication. The device design consists of three layers: a 100 μm needle buffer layer, a 200 μm needle guide layer, and a 300 μm hydrogel reservoir layer. The needle guide layer helps to keep the needle straight in the channel while the needle buffer layer prevents the needle from touching the bottom of the device. This is essential because cell contact to the plastic or glass device can alter cell behavior.

The microfluidic device mold was fabricated by depositing and exposing three subsequent layers of SU-8 onto a silicon wafer using two fabrication methods: the reverse (Figure 2) and upright (Figure 3) SU-8 processes. Each SU-8 layer of the device design was exposed to UV light using the ABM contact aligner and after all layers were exposed, the wafer was developed.

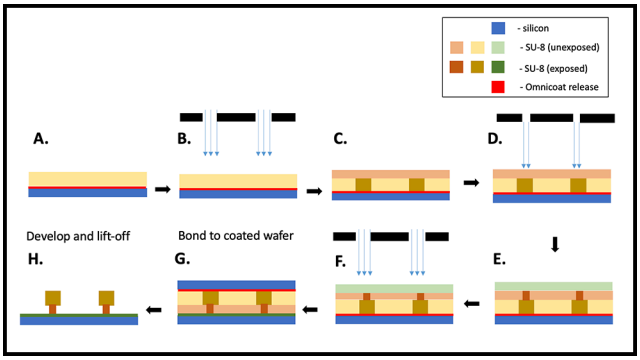

Figure 2: The reverse SU-8 fabrication process steps: a) Coat the wafer with OmniCoat® and SU-8. b) Expose at 350 mj/cm². c) Coat the wafer with a second layer of SU-8. d) Expose devices from 150-600 mj/cm² in 50 mj/cm² increments. e) Coat the wafer with a third layer of SU-8. f) Expose at 450 mj/cm². g) Adhere second wafer to first wafer. h) Develop wafer sandwich and dissolve OmniCoat to remove the first wafer.

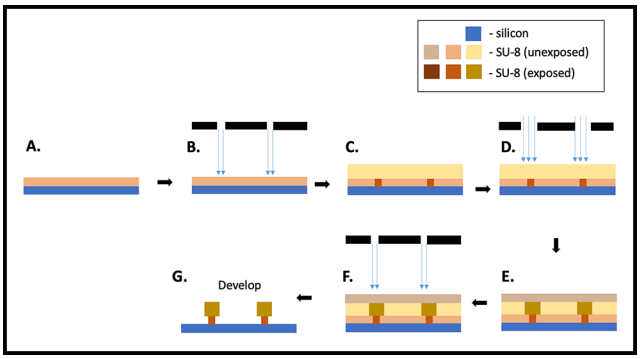

Figure 3: The upright SU-8 fabrication process steps: a) Coat the wafer with SU-8. b) Expose at 300 mj/cm². c) Coat the wafer with another layer of SU-8. d) Expose devices at 150-600 mj/cm² in 50 mj/cm² increments. e) Coat the wafer with a third layer of SU-8. f) Expose at 450 mj/cm². g) Develop wafer.

Device Characterization. The devices were sent to the Biotechnology Resource Center at Cornell University where they were characterized using a CT scanner. The CT scan image shows the devices made from both reverse and upright SU-8 processes. The reverse SU-8 process produced the necessary overhang in the needle guide layer, though slightly elongated, while the upright SU-8 process produced no overhang at all (Figure 4). It was found that the needle buffer and guide had a thickness of about 418 μm, which was close to our estimated 400 μm, while the total thickness of the device is around 745 μm, slightly larger than the 600 μm we had proposed.

Conclusions and Future Steps:

The next phase of this project is to finalize the device fabrication process in order to maximize the effectiveness of the devices. Then, we will cast the PDMS device, insert needles into the channels to fill the collagen reservoir, fill the media reservoirs and lastly seed the channels with tumor and endothelial cells (Figure 1). To increase the strength of the bond between the wafer and substrate, and minimize loss of devices during wafer transfer, we plan to use a substrate bonder. By using metabolic gradient experiments and completing cell culture experiments in our devices, we hope to learn more about the behavior of CSCs and how their properties are affected by the perivascular niche.

Acknowledgements:

Special thanks to Matthew Tan, Claudia Fischbach, Melanie-Claire Mallison, and the CNF Staff. This work was performed at the Cornell NanoScale Facility, as part of the 2021 Research Experiences for Undergraduates (CNF REU) Program, funded by the National Science Foundation via the National Nanotechnology Coordinated Infrastructure (Grant No. NNCI-2025233).

References:

[1] SEER Cancer Stat Facts: Female Breast Cancer. National Cancer Institute. Bethesda, MD, https://seer.cxancer.gov/statfacts/html/breast.html.

[2] Yoo, Y. D., Han, D. H., Jang, J. M., Zakrzewska, A., Kim, S.Y., Choi, C.Y., Lee, Y. J., and Kwon, Y. T. (2013). Molecular characteristics of cancer stem-like cells derived from human breast cancer cells. Anticancer research, 33(3),763-777.

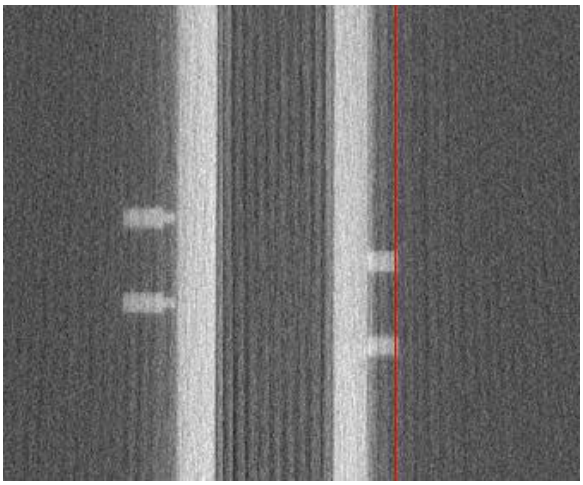

Figure 4: The CT scanner image of the devices from both the reverse (left) and upright (right) SU-8 fabrication methods.