# Matrigel Co-Flow at the Qi Lab Overview

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**Introduction:**

One section of the Qi lab focuses on modeling the human retina outside in vitro, or outside of the body. The retina is comprised of the inner and outer blood retinal barrier, which each regulates the flow of blood and consequently nutrients [1]. These barriers are comprised of six total layers of cells and proteins that are significant to glaucoma and other progressive retinal diseases [2]. Figure 1 is an illustration of the layers and order of the retina. The primary setback to treating retinal disease has been the difficulty in modeling it, as collecting in vivo data is nearly impossible without permanently damaging the subject [3]. The current method of modeling utilizes microfluidics, or micrometer scale pipes that enable cells to interact with other pipes. Figure 2 is an image of our classic three channel chip that we used for over 80 experiments and counting.

**Objectives:**

There are two identified areas of improvement for these devices. First, our current models only include three cell types: endothelial, microglia, and T-cells. Additionally, the PDMS, used to create gaps between rows of cells, is too thick and does not properly mimic the distance between cell cultures. The human retina is on average about 250 micrometers, while the current design uses 150 micrometers per cell type [4]. One of my main projects, the co-flow project, aims to flow each layer of the blood retinal barrier into a singular 250 micrometer channel utilizing laminar flow and polymerization in a Y-junction pattern to keep the flows of cells separate but able to interact in a much closer area.

**Methods:**

PhD student Andrea Goertzen and I worked on this project together: she worked on creating a device that minimized turbulence in the chip while I worked on the application and testing of the devices. The first project was creating a method to reuse chips to save resources. Traditionally trypsin was washed through channels which got rid of cells but not polymer residue, which is hard to see without adding a dye. I created and tested a new solution that was half trypsin and half EDTA solution which nearly instantly dissolved the polymers as well. I also made a protocol and optimization process to cut the experiment duration from an hour to twenty minutes. The procedure is split into five sections: priming and preparing the chip, making dyes, preparing Matrigel solutions, performing co-flow, and preparation for repeats.

**Results:**

Before implementing six layers of cells, we decided to test two layers of Matrigel with different dye solutions; Figure 3 is a result. Matrigel is a polymer solution that includes many hydrogels present in our bodies, which is advantageous for validity and efficacy [5]. Matrigel polymerizes, or hardens, at 10 degrees Celsius, so the polymerization process was simple if temperature conditions were met. Unfortunately, we found that as Matrigel solidifies it does so unevenly, causing mixing in the channel. Additionally, one flow sometimes polymerizes faster than the other, which causes one solution to be flooded out. Other similar styles of co-flow only utilize one polymerized area and enable flow to continuously occur on adjacent sides, which assists in maintaining laminar flow [6]. Thus, our style of polymerization and subsequent issues are unique to the project. We hypothesized that heat transfer and the ununiform structure of Matrigel were the cause of the issue, but did not have time to continue testing with other temperature or hydrogel conditions. My last project was creating a data spreadsheet of different polymerization times of different Matrigel solutions (mostly mixed with dyes) and temperature conditions. This gave insight into how different durations of the steps of the co-flow set up affected polymerization times. The hope of the project is to interpolate data and predict how long polymerization will take in future projects.

**Conclusions and Recommendations:**

Although the co-flow project was not initially a success, our team learned about common pitfalls faced when dealing with polymers. In the future if anyone returns to the project, I suggest two potential solutions. First, UV polymerization is a viable option instead of temperature controlled polymerization so long as it is optimized [7]. This allows for a much more controlled environment, and time constraints at certain temperatures are no longer a variable. Second, a different polymer such as gelatin, collagen, or GelMA could be used to establish a more uniform layer of cells, as they are more uniform in nature than Matrigel [8]. In the time that has passed since the project, I have gained tremendous amounts of knowledge about polymers and fluid dynamics and believe that this is still a path that leads to a better understanding of retinal diseases.

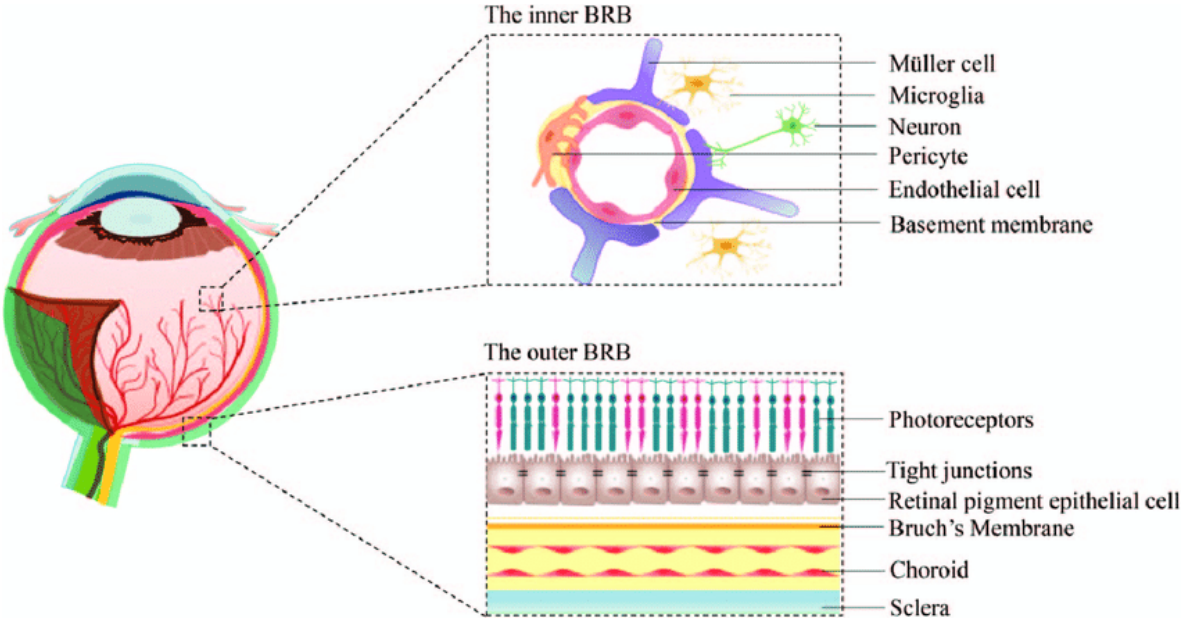


Figure 1: Outer and Inner blood retinal barrier diagram [1]

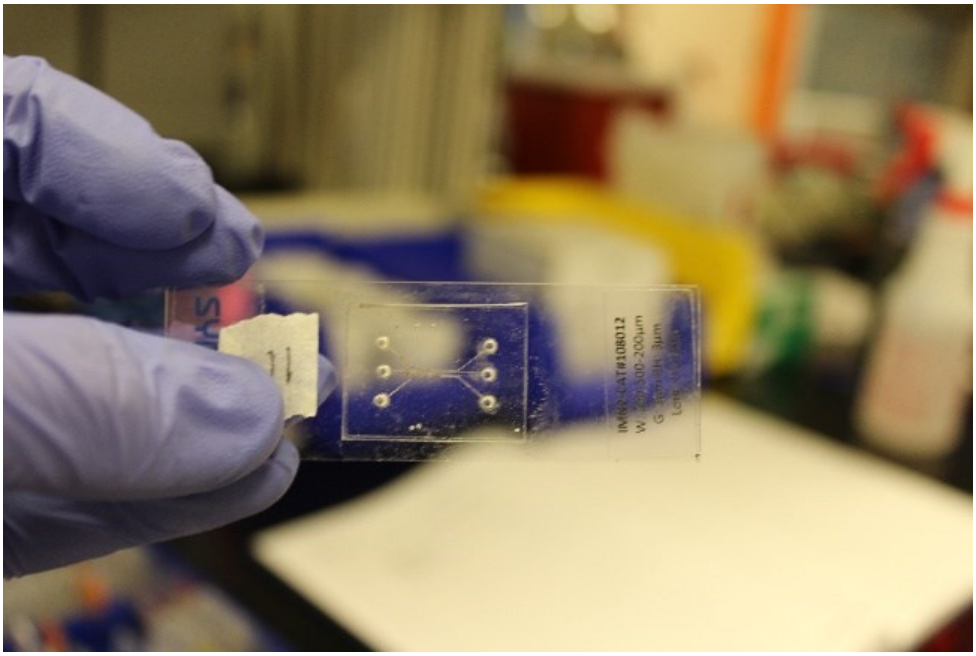

Figure 2: A classic three-channel microfluidics chip

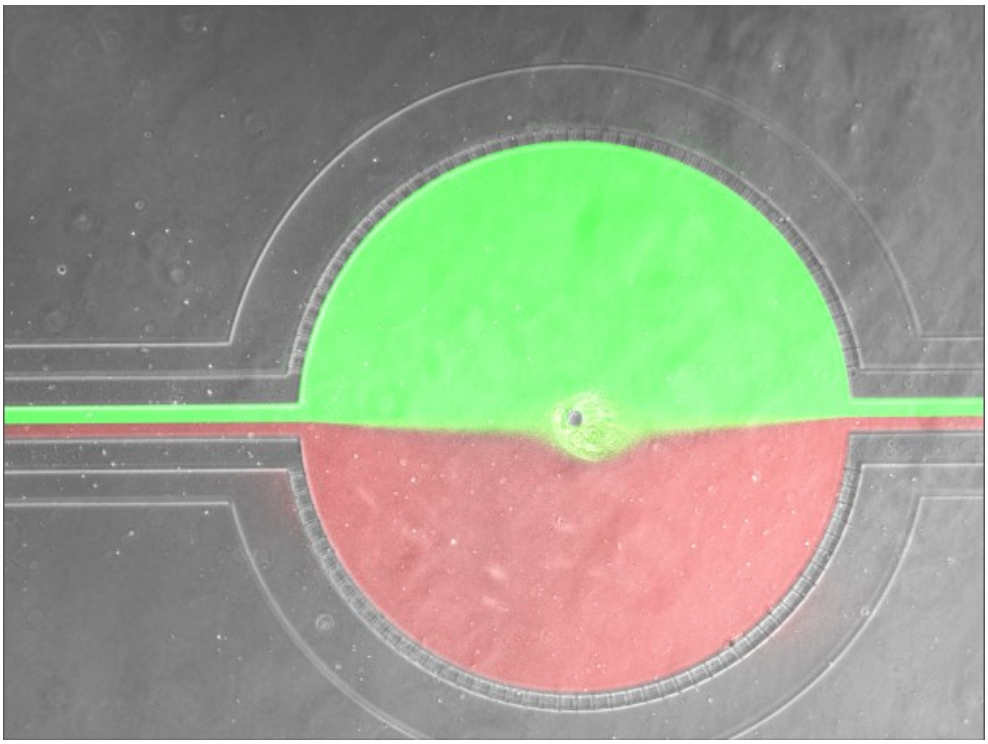

Figure 3: Dyed Matrigel in flow before Polymerization

**References**


[1] M. Campbell and P. Humphries, "The blood-retina barrier: tight junctions and barrier modulation," (in eng), *Adv Exp Med Biol,* vol. 763, pp. 70-84, 2012.

[2] X. Yang, X.-W. Yu, D.-D. Zhang, and Z. Fan, "Blood-retinal barrier as a converging pivot in understanding the initiation and development of retinal diseases," *Chinese Medical Journal,* vol. Publish Ahead of Print, 08/25 2020, doi: 10.1097/CM9.0000000000001015.

[3] M. Radisic, "Building a better model of the retina," (in eng), *Elife,* vol. 8, Oct 4 2019, doi: 10.7554/eLife.51183.

[4] A. Chan, J. S. Duker, T. H. Ko, J. G. Fujimoto, and J. S. Schuman, "Normal macular thickness measurements in healthy eyes using Stratus optical coherence tomography," (in eng), *Arch Ophthalmol,* vol. 124, no. 2, pp. 193-8, Feb 2006, doi: 10.1001/archopht.124.2.193.

[5] A. Passaniti, H. K. Kleinman, and G. R. Martin, "Matrigel: history/background, uses, and future applications," (in eng), *J Cell Commun Signal,* vol. 16, no. 4, pp. 621-626, Dec 2022, doi: 10.1007/s12079-021-00643-1.

[6] E. Rosellini and M. G. Cascone, "Microfluidic Fabrication of Natural Polymer-Based Scaffolds for Tissue Engineering Applications: A Review," *Biomimetics,* vol. 8, no. 1, p. 74, 2023. [Online]. Available: https://www.mdpi.com/2313-7673/8/1/74.

[7] D. Nieto, J. A. Marchal Corrales, A. Jorge de Mora, and L. Moroni, "Fundamentals of light-cell-polymer interactions in photo-cross-linking based bioprinting," (in eng), *APL Bioeng,* vol. 4, no. 4, p. 041502, Dec 2020, doi: 10.1063/5.0022693.

[8] B. Zhang, Y. Luo, X. Zhou, L. Gao, X. Yin, and H. Yang, "GelMA micropattern enhances cardiomyocyte organization, maturation, and contraction via contact guidance," (in eng), *APL Bioeng,* vol. 8, no. 2, p. 026108, Jun 2024, doi: 10.1063/5.0182585.