Motivation: Adult stem cells are a promising candidate for stem cell therapies as they would be derived from a patient's own tissue, lowering the risks of bodily rejection associated with stem cell therapies[1]. The challenge, however, is that adult stem cells exist in extremely low numbers amongst other cell types. The conventional means of cell sorting involves using known surface-expressed proteins as labels to sort by, but this knowledge simply does not exist for many adult stem cells, including retinal stem cells (RSCs)[2]. To combat this problem, I plan to explore cell sorting using a label-free microfluidic platform, namely a device which uses microscale fluid physics to sort cells based on physical phenotypes. To efficiently isolate adult stem cells, a device would require sensitivity in sorting through a heterogeneous population of multiple cell types, the ability to discriminate the adult stem cell of interest, and to verify the correct cell has been found. To the best of my knowledge, no solution exists which can perform all the aforementioned tasks on a single device. *I propose to design and build a "rare stem cell discovery" platform, namely a label-free microfluidic device which isolates adult stem cells by their size and subsequently captures them based on levels of polarizability.*

Background: Our lab at the University of Toronto specializes in rare cell capture using microfluidic devices, having previously demonstrated capture of circulating tumour cells (CTCs) present in blood, existing as ~10 cells per mL of blood, with over 90% efficiency[3]. Having demonstrated proficiency in rare cell capture, our lab amongst four other collaborators began to study retinal stem cells (RSCs) under funding by Medicine by Design. RSCs are an adult stem cell type which comprise <0.05% of retinal cells[2]. Standard cell sorting techniques, including fluorescence-activated cell sorting (FACS), have only been able to purify RSCs from the initial 0.05% to 0.2%[2], due to the absence of known unique RSC labels and cell death in the machine. Using the RSC model as my application, I will design a device based on *two different label-free sorting parameters*: size and polarizability. I explored the first parameter during my first year of graduate studies this past year, designing devices to sort retinal cells into multiple size ranges. I have shown the distribution of RSCs are 10-15 μm, relative to larger and smaller retinal cells, with initial data showing purity already 10-fold greater than FACS. I now propose a subsequent sort using a second label-free technique called dielectrophoresis (DEP). DEP sorts cells based on their membrane polarizability, with previous studies using the technique to separate stem cells from their differentiated progeny[4,5,6]. Using RSCs, my research question is to ask whether size-sorted adult stem cells can be efficiently sorted using DEP. My rationale is that cell polarizability is partially a function of cell size[7], so if I only probe cells of a certain size, DEP will become sensitive to polarization differences between cell types instead of cell sizes. I hypothesize that within a population of equally sized cells from primary tissue, adult stem cells can be discriminated using DEP.

Device Concept: My proposed device has two stages (Figure 1). The first stage is a size sorting region to isolate a size range previously determined to contain the highest population of the stem cell of interest, which I have already done for RSCs. Size sorting will be done based on a technique called deterministic lateral displacement (DLD)[8], sorting cells above and below a designed size. I will cascade two DLD regions such that only a narrow range of cell size (varying by ±1um) will flow to the next stage. The second stage includes ten straight channel zones filled with DEP traps, namely electrode configurations that can hold single cells in suspension based on DEP force[9]. Each zone increases in width because, with a constant inlet flow rate, increasing width decreases the linear flow velocity within the chip. This makes the latter zones' DEP traps more sensitive to weak dielectric signals, meaning this device can capture cells at ten distinct levels of cell polarizability. Our lab has previously demonstrated the "zone" concept to capture CTCs antibody-tagged with magnetic nanoparticles using magnetic traps[10], but I now aim to use zones in a novel, label-free modality. By trapping individual cells within hundreds of DEP traps in each zone, this device is a novel platform for on-chip sphere forming, a cell culture method used to identify stem cells where only stem cells divide into free-floating "balls" of cells. The specificity of sphere forming assays is limited since other cells in culture can aggregate into artificial spheres[11], but this issue is avoided in my proposed chip since single cells are individually trapped. If stem cells can be sorted according to their polarizability, I expect spheres will only form within one or few zones. Once the zone distribution of spheres is known for a certain stem cell type, sphere forming will not be necessary in subsequent sorts and the stem cells can be extracted from their respective zones.

Aims: My role in this project is to design and build the proposed device, and I have three aims:

Aim 1: I will design and simulate device geometries for the size sorting stage and DEP trap stage. Having done size sorting of retinal cells over the past year, I have already performed fluid flow simulations of size sorting devices to quantify the performance of devices with different geometries. The next step will be to optimize design geometries to account for cell deformably since cells can behave smaller than they actually are under fluid flow[12]. For the DEP trap stage, electrode specifications (e.g. height, cross-sectional shape, the number needed per trap) will be optimized to create a negative electric field gradient towards the trap's centre. Since many traps will exist in close proximity to each other, simulations will be performed to reduce voltage crosstalk and noise. This analysis requires knowledge of computational electromagnetism, a field in which I have experience from my undergraduate studies, including finite difference methods for solving Maxwell's equations. These studies are necessary to verify the theoretical approaches being taken, and to account for non-idealities to be encountered in the real device.

Aim 2: I will optimize device fabrication and material composition of the device to allow for cell culture. Standard photolithography techniques can be used for both stages of the chip, with features made of negative photoresist for the first stage and thin-film metal deposition for traps in the second stage. The chips can be sealed with a silicone or glass layer. I will consider integrating heat sinks in my device to dissipate local heating from the electrodes. I will determine an optimal material coating for the chip to prevent adherence of cells to the chip to conduct sphere forming assays. The number of electrical interconnects per zone will be minimized to minimize fabrication challenges. These sets of optimizations are necessary to ensure consistent fabrication and a suitable microenvironment for cells.

Aim 3. I will test the sorting and capture efficiency of the chip using the RSC mouse model. I will optimize inlet flow rate and input cell concentration to ensure the efficiency of the cell sorting stage. The frequency of the DEP trap AC voltage will be tuned according to iterative experiments of capturing cells and conducting sphere forming assays while cells are trapped. Frequency tuning experiments are necessary to discover at what frequency the majority of RSCs will be captured. With these sets of studies, a protocol can be developed for efficient stem cell discovery.

Potential Challenges: In practice, cells larger or smaller than expected may find their way to the DEP trap region and influence the cell distribution. A possible solution will be to repeat the size sorting multiple times by adding more identical DLD arrays to correct sorting errors. Another challenge I may face is my device not having high enough resolution to fraction out the RSCs. A possible solution is to increase the number of zones on my device to make it more sensitive to smaller changes in polarizability. While fabrication may be more challenging, our lab has previously demonstrated microfluidic devices with 100 zones[3].

Significance: Creating a device to sort adult stem cells without the use of labels is critical to advance stem cell research. For example, retinal cell transplantation presents a viable mechanism to restore lost vision for patients with degenerative eye disease[13], but successful isolation of high purity RSCs is first required so our collaborators can build these therapies. I also see the utility of my device spanning past adult stem cells for use in my lab, such as in providing a platform to profile the distribution of CTCs as a function of physical phenotype, an area which is largely unexplored. The success of my project will open avenues to isolate adult stem cells when little is known about the cell type's composition, making my device a powerful tool for stem cell discovery.

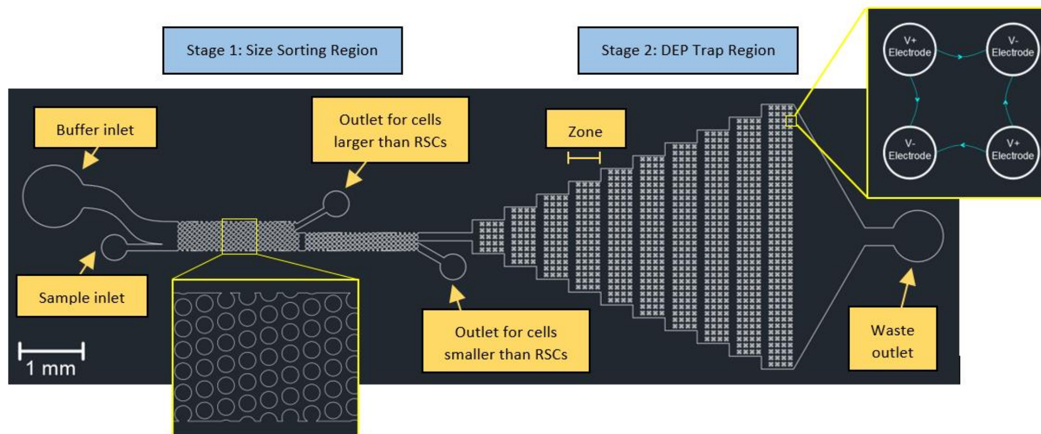

Figure 1. Concept of my proposed device

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