Research Proposal Example 2

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. The challenges in obtaining these cells, however, is that they are rare and little is known about their surface-expressed proteins (i.e. labels), making conventional cell sorting ineffective. Microfluidics is an emerging engineering sub-discipline that has been used for label-free cell sorting, using microscale physics principles to manipulate low concentrations of cells. I propose to build a novel, label-free microfluidic device which isolates adult stem cells by their size and subsequently captures them based on levels of polarizability.

Rationale: Using mouse retinal stem cells (RSCs) for my model, I previously designed a device to sort RSCs based on size (refer to "Thesis Information"). I now propose to integrate a secondary sorting stage on my device using dielectrophoresis (DEP), a label-free technique which distinguishes cells based on their membrane polarizability using an inhomogeneous electric field. Previous DEP studies have demonstrated polarizability differences between stem cells and their differentiated progeny[1]. Since polarization is partially a function of *cell size*, the initial size-sort on my device will increase DEP sensitivity to differences between *cell types*. My device will employ DEP traps, namely electrode configurations that use DEP force to attract and hold single cells[2]. Arrays of DEP traps will be patterned into many zones on the device, with each zone addressed at a different AC voltage frequency, making this a novel device to capture cells at multiple levels of membrane polarizability. By capturing cells, my device is a robust platform for on-chip sphere forming, a technique of verifying the presence of stem cells since they divide into a "ball" of cells when cultured. Conventional sphere forming assays can be misleading since other cells in culture can aggregate into artificial spheres[3], but my device avoids this since individual cells are trapped. I hypothesize that within a population of size-sorted cells, adult stem cells can be discriminated using zones of DEP traps, with spheres forming in only one or few zones.

Aims: *1. Conduct computer simulations of device geometries*. The size sorting stage will be optimized to account for cell deformability. If, in practice, cells out of the designed size range pass to the DEP trap stage, a solution would be to repeat the size sorting stage multiple times to correct the errors. For the DEP trap stage, I will simulate electrode configurations to reduce electrical interference between traps. *2. Optimize device fabrication and material composition of the device*. Standard photolithography techniques and metal deposition for electrodes will be used for the device. I will determine an optimal material coating for the device to conduct sphere forming assays, and integrate heat sinks to minimize electrode heating. *3. Test the sorting and capture efficiency of the device using the mouse RSC model*. I will optimize inlet flow rate and input cell concentration to ensure the efficiency of the cell sorting stage. The frequencies addressed to each zone will be tuned according to iterative experiments of capturing cells and conducting sphere forming assays while cells are trapped, to discover at what frequency the majority of RSCs are captured. If my device does not have high enough resolution to fraction out the RSCs, a possible solution would be to increase the number of zones.

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, but successful isolation of high purity RSCs is first required so our collaborators can build these therapies. The success of my project will open avenues to isolate adult stem cells, making my device a powerful tool for rare stem cell discovery.

References:

[1] Flanagan, L. et al. *Stem Cells* 26.3 (2008): 656-65.
[2] Voldman, J. et al. *Analytical Chemistry* 72.4 (2002): 3984-90.
[3] Pastrana, E. et al. *Cell Stem Cell* 8.5 (2011): 486-98.