Enabling gentle and rapid spiral microfluidic-based cell isolation via flow rate, antibody, and buffer optimization

Ali Ansari¹ and P.I. Imoukhuede, PhD¹
¹University of Illinois at Urbana-Champaign, Urbana, Illinois, United States of America

Motivation/Innovation: Cancer patients respond differently to therapeutics due to cell heterogeneity¹–³. Advancing personalized medicine requires isolating and profiling heterogeneity; however, current techniques are slow⁴ and may disrupt biomarkers⁶,⁷,¹⁰–¹³. Thus, gentle and rapid cell isolation is necessary¹³; towards this aim, we optimize our Secondary Anchor Targeted Cell Release (SATCR) system¹⁴ to spiral microfluidics (Fig. 1A).

Methods: We simulate spiral wall shear stress (WSS) with FEAP software (Fig. 1B). We integrate SATCR and spiral microfluidics by fabricating a polydimethylsiloxane (PDMS) spiral mold (Fig. 1C), using 3D printed masters, and bond to glass via oxygen plasma. Human umbilical vein endothelial cells are fixed with 4% formaldehyde and captured via biotinylated CD31 antibodies. Cells are released via PBS-based biotin buffer. We measure buffer osmolarity with the Wescor Vapor Pressure Osmometer Vapro 5520, and cell diameters/concentrations with the Countess II Automatic Cell Counter.

Results: We report four key accomplishments: (1) Flow rate: we determine gentle flow conditions, simulations reveal that 300 µL/min gives 0.1 mPa mean WSS (Fig. 1B), one order of magnitude below arterial WSS¹⁵. (2) Degree of freedom: we determine that release solution concentrations can be varied without disturbing cells: a broad biotin concentration range, 0-4 mM, does not alter buffer osmolarity (Fig. 1D) nor cell diameter (data not shown), our metric for gentleness, offering a significant degree of freedom in device optimization. (3) Antibody optimization: we improve cell capture via a modified antibody, increasing cell isolation efficiency by > 25% compared to a non-modified antibody (Fig. 1E). (4) Buffer additive: we determine that an optimal [poly-L-lysine (PLL)] = 0.05-0.5 mg/mL, stabilizes cell diameter, thus enabling gentle cell isolation. Optimal PLL concentrations cause no cell diameter change (Fig. 1F). Conversely, without PLL, cells experience a cell diameter decrease > 25% (p<0.001, ANOVA Fisher test, Fig. 1F) and buffer shows a pH change: pH_in = 7.2 and pH_out = 6.4. We hypothesize that PLL stabilizes anion sequestration occurring due to the acidic streptavidin (SAv) isolectric point. Future studies will explore SAv replacement with neutravidin (NAv), a variant with neutral charge, low non-specific interactions, and similar biotin affinity¹⁵.

Conclusions: We optimized SATCR spiral microfluidics for cell isolation, identifying a flow rate with low WSS, identifying biotin-mediated release concentrations can be varied without cell disruption, optimizing our antibody, and determining that PLL supplementation stabilizes cell diameter. These optimizations will enable the gentle and rapid cell isolation needed for personalized cancer medicine.
Figure 1: SATCR System Schematic (A) Computational wall shear stress simulation in spiral design (B) Spiral mixer design with scale bar of 33 μm. (C) Biotin titration and osmolarity. (D) Comparison of cell pull-down for hybridized and constituent antibodies. (E) Cell Diameter and PLL titration (F)
REFERENCES:


