Supplementary Material (ESI) for Lab on a Chip

Cell Viability Test

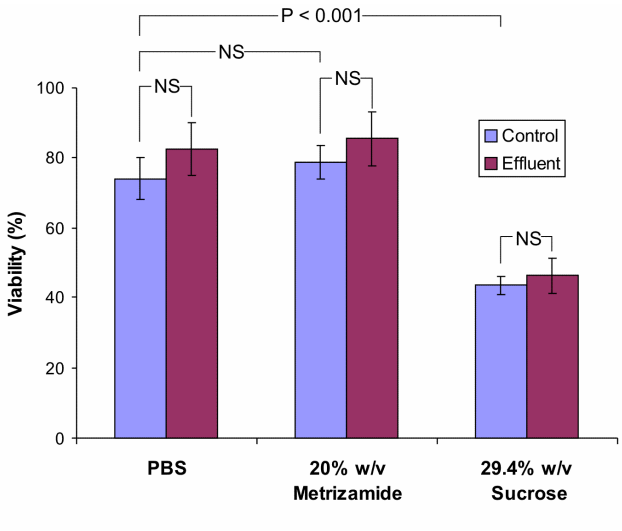
Materials and methods

Human umbilical vein endothelial cells (HUVEC) were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma Co., St. Louis, MI, USA) with D-glucose at 4.5 g/L, 10% FBS, HAT (100 µmol/L hypoxanthine, 0.4 µmol/L aminopterin, 16 µmol/L thymidine), 1000 U/L penicillin, and 1 mg/L streptomycin. The cell cultures were maintained at 37 ºC in a humidified atmosphere of 5% CO_2 and 95% air. Before experiment, cells were trypsinized and resuspended at a final concentration of 2.5×10^6 cells/ml into three solutions – PBS, metrizamide (20% w/v metrizamide dissolved in 31% PBS and 69% DI water to make the final osmolarity 290 mosm and density = 1.11 g/cm^3), and sucrose in deionized water (29.4% w/v, osmolarity > 800 mosm, density = 1.11 g/cm^3). The prepared cell suspensions were then added with 2 mM of calcein-AM and 4 mM of EthD-1 (Invitrogen, Carlsbad, CA) for staining live and dead cells, respectively. 100 µL of each cell suspension was processed with the same PDMS sorting device for the beads sorting experiment but run at 100 times faster (Reynolds number~0.3) to enlarge the possible effect of a MVM device on cell viability. The device-processed and non-device-processed cell suspensions were collected, placed on a hemocytometer, and imaged for counting viable and dead cells using fluorescence microscopy.

Image analysis

The fluorescence images were analyzed using an image processing program (ImageJ, National Institutes of Health). Briefly, background subtraction, thresholding followed by particle analysis was applied to count viable and dead cells automatically. Data were then analyzed using t-test to determine statistical significance (SigmaStat, Systat Software Inc., Chicago, IL).

Figures and Captions



***SI Figure 1**: Viability of cells in various solutions. HUVEC were used to determine the viability of cells after flown through a MVM device in three different solutions- PBS, 20% w/v metrizamide, and 29.4% w/v sucrose solutions. The HUVEC viability was determined by using the LIVE/DEAD Cell Viability/Cytotoxicity Kit. The source and effluent of cell suspensions were collected and imaged by an inverted microscope. Viable and dead cells were automatically counted using an image processing program (ImageJ, National Institutes of Health). Data were analyzed statistically using SigmaStat. NS: statistically nonsignificant, n=5. The blue and purple bars represent data from non-device-processed and device-processed cells respectively.*