Automated Processing of Human Blood Leukocytes by a Plastic Disposable Microfluidic Deterministic Lateral Displacement Device

Tony Ward1, Alison M. Skelly1, Khushroo Gandhi1, Zendra Lee1, Christopher R. Dosi1, Myra Koedjijo1, Tanisha Saini1, Joseph L. D’Silve2, Yu Chen3, MinJung Kim3, James Moynihan3, Xiaochun Chen1, Lee Aurich1, D. Recktenwald3, R.H. Austin1, Mike Grisham1, Curt Ivins1, James C. Sturmi1

1 GSB Scientific LLC, 600 East Lehigh St., Richmond, VA, 23229, 2 Princeton Institute for the Science and Technology of Materials, Department of Electrical Engineering, Princeton University, Princeton, NJ 08544, 3 Center for Stem Cell Biology & Regenerative Medicine and Greensleaves Cancer Center, Departments of Physiology and Pediatrics, University of Maryland School of Medicine, Baltimore, MD, 21201, 4 Delsys LLC, F-Box 12560, Bozeman, MT 59760-7 Princeton Institute for the Science and Technology of Materials, Department of Physics, Princeton University, Princeton, NJ 08544

Summary

Multi-parameter flow cytometry is increasingly viewed as a powerful tool in research and clinical applications, driving a growing need for improved efficiency in the area of sample preparation. Current sample preparation methods are labor and time intensive and involve substantial cell losses, typically associated with multiple centrifugation steps. These manual time consuming steps typically lose between 10-30% of cells as a function of which step is involved, frequently resulting in >25% cell loss in complex protocols, and with sometimes preferential loss of specific cell types. Our previous work in a silicon device showed that Deterministic Lateral Displacement (DLD) or “bump array” technology is capable of separating particles on the basis of size. Here, we present the reduction to practice of the DLD approach using a single channel high precision plastic microfluidic device.

DLD Microchip Device

Processing via DLD involves pumping 1:1 diluted blood through a specially designed array of microposts under laminar flow conditions in a microscope slide sized chip. The design has a critical dimension of 4.5µm to separate and recover WBCs. Sample enters at the outside edge(s) of the array and cells above a target size are gently deflected by the microposts into a clean stream of waste respectively. Single channel schematic shown.

Effective Recovery of Viable Cells >6µm

To test the ability of the Microchip to discriminate smaller cells of interest from erythrocytes, CMTM labelled mouse Splenocytes were used and counted using the Coulter principle (A). Labelled cells (B) were spiked into whole blood at 20,000/ml and processed normally, revealing recovery of spiked cells in the DLD cell product (C-D). Cell viability was determined using Propidium Iodide. A fluorescence threshold that contained CD45 APC-Cy7 and reference beads (Black) was used to eliminate potential for dead cells appearing below a typical FSC threshold. DLD is slightly cleaner because of removal of small debris.

DLD was functioning as predicted to recover viable cells 6um and greater in size.

Selected References


Superior WBC Recovery vs Lysis & Ficoll

Whole blood from 3 normal donors were red cell depleted via ammonium chloride lysis, Ficoll, or 24 channel DLD Microchip to assess cell processing losses (2.0 ml per protocol). Lysis protocols were performed with 1 or 2 wash steps to accommodate manufacturer’s protocol variation. Triplicate determinations of WBC and PBMC counts were determined by flow cytometry using a nucleic acid dye (DAPI) and absolute count reference particles (ebioscence). Input WBC counts from whole blood were obtained using a 1:400 dilution and a fluorescence threshold that included both reference particles and nucleated cells in ~1.2 ratio. Light scatter and DAPI gating were used to determine PMMC content of cell inputs.

DLD Microchip WBC recovery was >99% compared to Lysis using standard protocols which can lose in excess of 35% in a 1 wash protocol and >50% in a 2 wash protocol. Even when a conservative aspiration to include all possible PMMC cells was used, a standard Ficoll protocol lost almost 50% of input PBMCs. The input PBMC portion of the blood in this study ranged from 18-43%, spanning typical values.

DLD recoveries all major WBC subsets effectively

Microchip processed blood was compared to lyed ns wash processing and diluted whole blood control.

Cells were stained with CD3/CD19/ CD45 after processing. 3µM DRAQ5 and 12count reference beads (ebioscence) were added to normalize analyzed volumes in microchip product and lyed controls. Data was collected using a forward scatter threshold to allow erythrocyte contamination in the traditional gating region.

Column I shows traditional scatter profiles, Column II shows RBC/WBC to bead relative percentages, column III shows light scatter following a DNA gate, and column IV shows traditional lymphocyte subset analysis. The waste fraction shows essentially zero WBC, and the product shows recovery of major subsets with comparable staining and a 5 fold better WBC/RBC ratio versus the lyse no wash protocol.

Efficient Depletion of RBCs and WBC Recovery

Initial protocols used a bolus of air to evacuate the microchip at the end of a run (Air Push) and achieved >99% depletion of RBC. On inspection, we found that a small dead volume upstream of microchip in fluidic connectors was impacting RBC depletion rates.

A second series, addressing dead volume was run to evaluate the true performance of the DLD (Buffer Push). This resulted in ~10 fold improvement, achieving 99.985% average RBC depletion in ~30% less time than a conventional 1 wash lysis procedure and required no hands on steps. Further the DLD cell product stream can be fed directly to a flow cytometer without further processing.

Conclusions

RBC Depletion rates of 99.985% combined with efficient and unbiased recovery of WBCs with minimal hands on time has been demonstrated with plastic DLD microchips. Their performance has significantly exceeded the level achieved by current approaches.

• Does not adversely affect cell viability
• Faster performance
• Optimal approach for rare cell enrichment and analysis
• Commercially viable high performance plastic consumable
• Scalable design offers significant simplification of workflows

These results indicate significant user benefits, and suggest their utility for multiple other applications.

DLD Separation

www.gpbscientific.com

GETTING PEOPLE BETTER

804.477.1676

#146 B10