Multiphysics innovation for high speed, high recovery, and high viability automated cell separation

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Silin Sa, Candice Liu, Michael Kempnich, Ethan Nguyen and Liping Yu | Applied Cells, Santa Clara, CA 95054

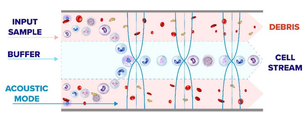
Introduction

Tumor biology, immunology, and immune-oncology from multi-omics at the single cell level have witnessed an unprecedented acceleration in recent years, with a major contribution of such speedup from new technologies and products bursting into the market. New technological solutions also contributed deterministically to the promise of cell-based therapy. However, conventional cell separation tools, such as density-gradient-based centrifugation, column-based magnetic cell separation, and even FACS (fluorescent activated cell sorting) have become limiting factors to broadening the application of single cell analysis from complex tissues samples and very rare cell types. In the development of cell therapy drugs, robust cell manufacturing platforms that are flexible, adaptable to various processes, and involve minimal human factors, are also widely sought after.

MARS® Cell Separation Platforms incorporate innovations in multiple cell separation technologies. The active acoustic cell separation does label-free separation at an unprecedented flow rate for any microfluidic device. Cells are moved in or out of clean buffers based on their physical parameters and the selection threshold is tunable. MARS® platform also offers a column-free in-flow magnetic cell separation technology, which allows a specific selection of cells based on their surface markers. The separation process can be done in a closed fluidic path in a fully automated version and has no capacity limit. MARS® are constructed as modular platforms to support a wide range of applications in the life science arena, including single cell analysis, precision medicine, as well as cell therapy.

Technologies and Platforms

MARS Acoustic Cell Separation



MARS Magnetic Cell Separation

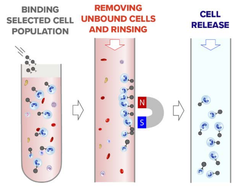


Figure 1: **Schematic illustration of the technologies** left, MARS® **high-speed** acoustic cell separation: cell suspension is pumped under laminar flow condition along with "wash buffer" in a microfluidic channel where acoustic standing wave is established. Selected cells move from input sample stream to buffer stream under acoustic pressure and cellular debris remain in the original sample stream. Right, MARS® **column-free matrix-free** magnetic cell separation: antibody conjugated magnetic beads are bound to specific cells and cells are pumped through a separation channel which is in proximity with magnetic source . Beads bound cells are pulled to wall of the channel and rest of the cells are pumped through. Once the sample is completed the channel is moved away from the magnetic source and separated cells are released automatically. The separation process can be done in continuous cycles with no capacity limit.

Figure 2: left **MARS® SP**, configured with three acoustic cell separation modules, is able to perform parallel cell wash, sequential cell separation, and media exchange; middle and right, **MARS® BAR**, configured with three magnetic separation modules, is able to perform parallel and sequential selection programs for positive and negative selections. The MARS® Bar offers tube- in- tube- out and bag –in- bag- out options.

Results

Acoustic Cell Separation – Granulocytes vs PBMCs

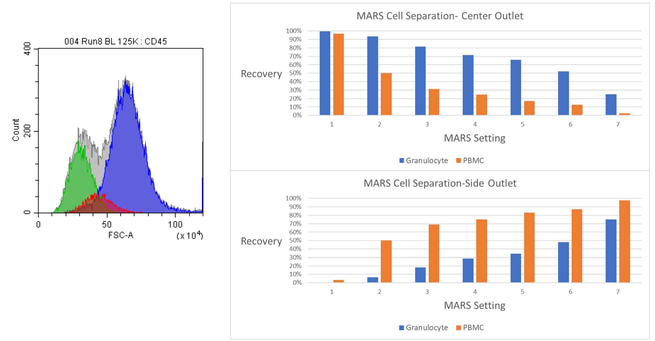


Figure 3, in whole blood granulocytes and PBMCs have different size parameters. When processed on acoustic device they can be isolated together from cellular debris (condition 1) or they can be enriched separately in the center outlet and side outlet of the acoustic device at varied amplitude. Flow rate: 0.8mL/min of whole blood with lysed red blood cells (lysis ratio 1:4).

Acoustic Cell Separation – Purification of Brain Nuclei

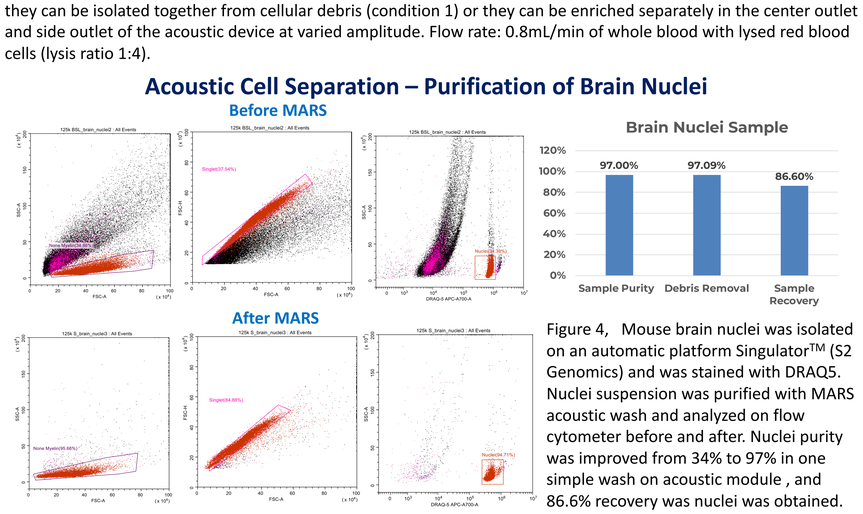


Figure 4, Mouse brain nuclei was isolated on an automatic platform Singulator™ (S2 Genomics) and was stained with DRAQ5. Nuclei suspension was purified with MARS acoustic wash and analyzed on flow cytometer before and after. Nuclei purity was improved from 34% to 97% in one simple wash on acoustic module , and 86.6% recovery was nuclei was obtained.

Rare Cells Enrichment with Positive Selection on MARS MAG

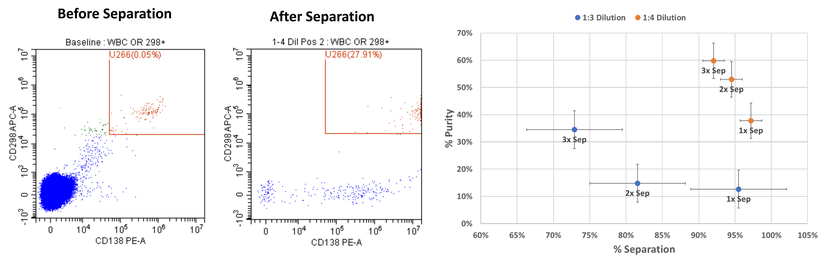


Figure 5. U266 cell line cells were pre-stained with CD298 APC and spiked in healthy donor's whole blood at frequency at 0.05% (left) and 0.03% (right) of all white blood cells. 0.05% sample was passing magnetic separation channel once at 1.0mL/min and spiked cells were enriched to 28%. The impact of dilution factor and **Serial** separation was evaluated with 0.03% spiked- in sample, and increased purity was obtained when positively selected cells were run through separation 2 times and 3 times at 1:4 dilution. Separation efficiency remained to be >90% at 1:4 dilution.

Results for Cell Therapy

Automated Large- Scale Isolation of Immune Cells

Target Populations in 1mL Leukopak Sample					
Cell Type	Live WBC	T Cells	CD8+ Cells	CD4+ Cells	B Cells
Count	88E6	54E6	16E6	34E6	12E6
% Live WBC	100%	61%	18%	39%	14%

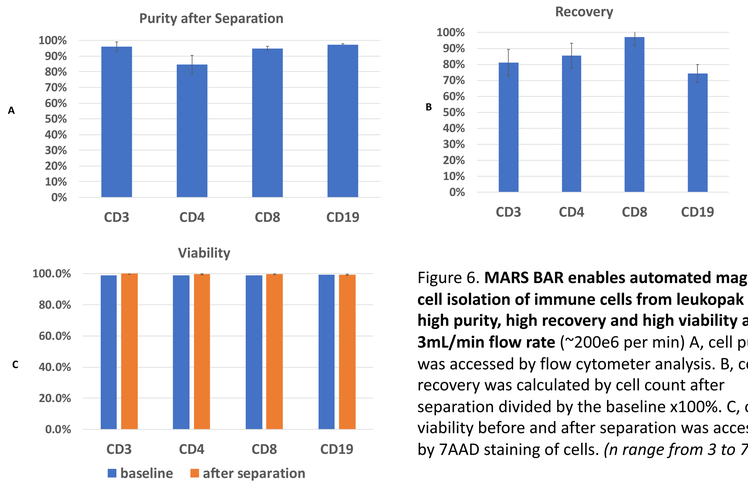


Figure 6. **MARS BAR enables automated magnetic cell isolation of immune cells from leukopak with high purity, high recovery and high viability at 3mL/min flow rate** (~200e6 per min) A, cell purity was accessed by flow cytometer analysis. B, cell recovery was calculated by cell count after separation divided by the baseline x100%. C, cell viability before and after separation was accessed by 7AAD staining of cells. *(n range from 3 to 7)*

Acoustic Cell Separation for Media Exchange

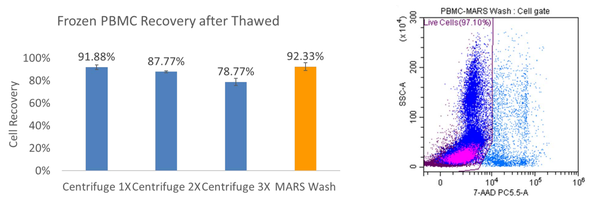


Figure 7. Cryopreserved PBMC was thawed, treated with DNase, and diluted 5x with PBS. The PBMC suspension was pass through MARS acoustic device and PBMCs were moved from DMSO containing media to clean wash buffer with high recovery (comparing to 3 times centrifugation process) and high viability.

Conclusions

- The innovations built in Applied Cells MARS® platforms provide automation for leukocytes separation, nuclei purification and rare cell enrichment for multi-omics cell analysis. The results demonstrate high recovery and high purity of cells.
- The large-scale Immune cell isolation has shown high purity, high recovery and high viability in multiple specificities; and high-speed media exchange with acoustic device is capable to replace conventional centrifugation-based process.
- The MARS® modular configuration and closed-fluidic option offer scalable and flexible solutions for cell therapy development.