A Novel Platform Providing Automated, Consistent and Gentle Cell Isolation from Tissues for Downstream Applications

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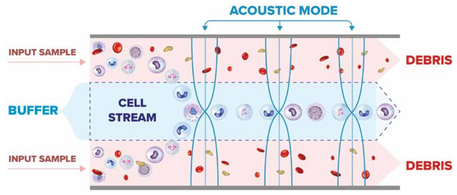
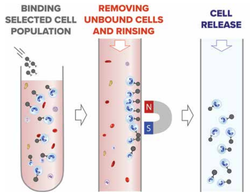
INTRODUCTION

Tumor-infiltrating lymphocytes (TILs) are lymphocytic cells that have invaded the tumor tissue. Adoptive cell therapy (ACT) of TILs is a strategy to modify the immune system to recognize tumor cells and carry out an anti-tumor effector function. Today, TIL therapies consist of ex vivo expansion of TIL from resected tumor material and adoptive transfer into the cancer patient. Although the treatments have shown promising results in various tumor types, the production and reactivity of TIL products from many solid tumor types is variable and requires further research. Therefore, an efficient way to isolate TILs is crucial for basic and clinical research applications. Conventional cell separation technologies such as Ficoll gradient centrifugation, column-based magnetic separation, and FACS single cell sorting have issues in low cell recovery, compromised cell viability, and are time-consuming.

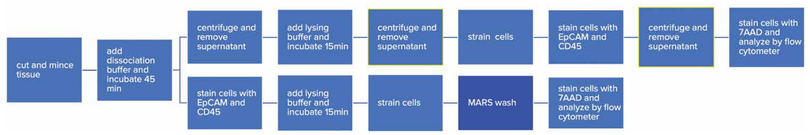
Here we present an easy and efficient immune cells separation method from a lung tumor biopsy sample using the novel cell separation platform --MARS®. MARS® platform employs two cell separation technologies: active-microfluidics acoustics as well as magnetic separation. Acoustic separation is based on the difference in physical parameters (size, etc.) and allows for the removal of lysed cell debris, dead cells, and other small particles. the MARS® magnetic cell separation technology performs a specific selection of tumor cells or immune cells. Both processes produce high purity and high recovery of target cells. The high-speed separation is controlled at low pressure and maintains TIL cell viability and functionality, so the isolated cells are ready for expansion.





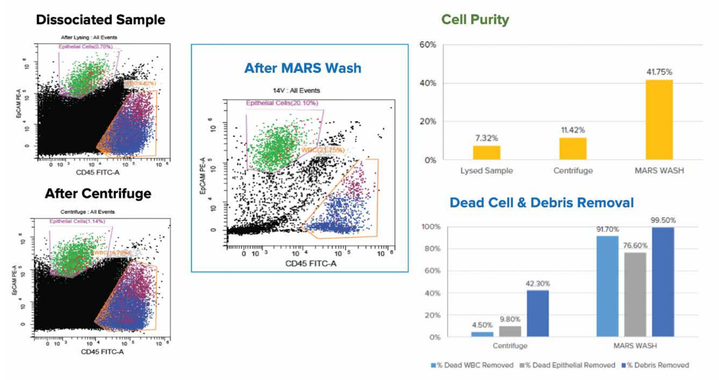
Schematic illustration of core technologies employed on MARS® cell separation platform. On the left, it is the MARS active-microfluidic acoustic cell separation. In a microfluidic channel, cell suspension and "wash" buffer flow through in a laminar format. When the acoustic field is on cells were pushed moving to the "wash" buffer under acoustic pressure, so cells and debris were collected at a different exit. On the right, it is the MARS column-free, matrix-free, capacity-limitless, inflow magnetic separation, which allows both positive and negative selection of cells based on the surface markers. Both processes are gentle to cells with no centrifugation, no pelleting of cells, and no high pressure. The processes were at high speed and demonstrate high purity, high recovery of cells with high consistency.

METHODS



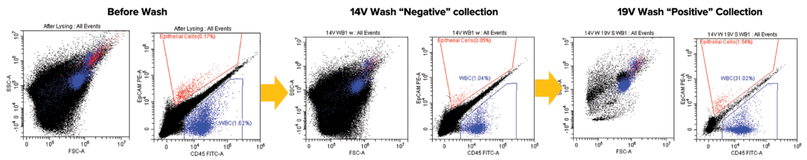
RESULTS

Acoustic Separation of Tumor and Immune Cells from Biopsy Dissociation



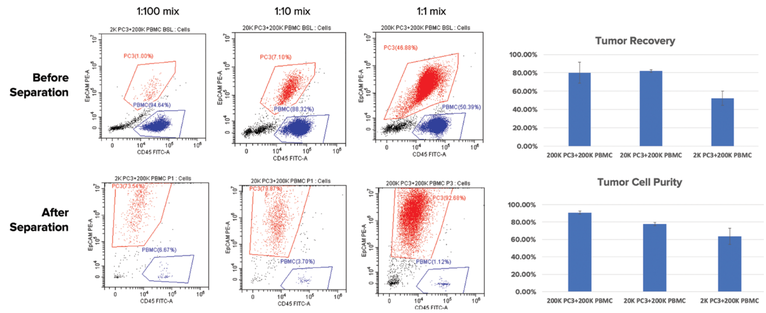
Tumor biopsy dissociated cell suspension was processed by centrifuge-wash for 3x and MARS acoustic wash at 0.8mL/min one pass. MARS wash achieved higher purity and cell recovery.

Two-Step Acoustic Separation to Enrich Tumor and Immune Cells Separately



Tumor biopsy dissociated cell suspension was processed by MARS acoustic wash at 0.8mL/min for two passes: first pass, a lower amplitude driving power was applied to the acoustic chip and ~70% tumor cells were enriched in the "positive" collection; the "negative" collection which contained 88% of immune cells and ~30% tumor cells was run at a second pass when a higher amplitude was applied. Through the second pass, immune cells were separated from debris and constituted 95% of all cells. Overall immune cell recovery was about 40%.

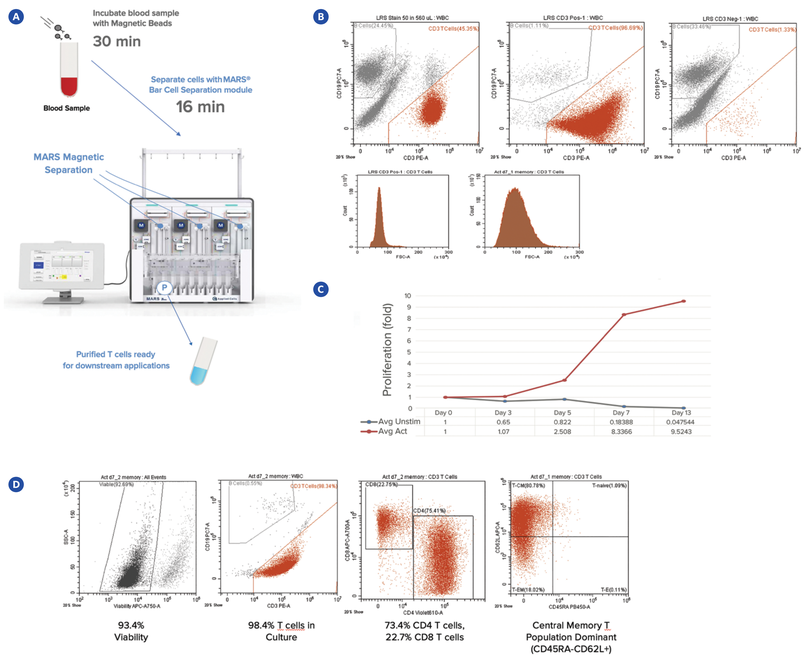
Acoustic Separation of Tumor and Immune Cells from Biopsy Dissociation



A mix of PBMC and PC3 cells mimicking tumor biopsy samples were stained with EpCAM-PE and antiPE magnetic nanoparticles (Applied Cells). The cell suspension was run through MARS magnetic separation channel at 0.5mL/min and magnetically captured cells were released automatically collected in the "positive" tube. PBMC went through a separation channel and was collected in the "negative" tube. At three different mixing ratios, the two samples with higher tumor percentage at baseline achieved ≥80% recovery and purity on average.

RESULTS

Large Scale Aseptic T cell Isolation from Apheresis Product Followed by Expansion



1 billion leukocytes collected by the apheresis process were handled aseptically through MARS magnetic selection of T cells (CD3+) (A). Cell count and phenotype were analyzed before separation, during activation, and expansion. After day 7, T cells were expanded almost 10 times and had 99,8% purity with the dominant central memory T cell population. (B, C and D).

CONCLUSION - MARS Family

3 Acoustic Modules	2 Acoustic +1 Magnetic Modules	3 Magentic Modules
UNTOUCHED SEPARATION	**BOTH TECHNOLOGIES**	**IMMUNO-MAGNETIC ISOLATION**