A New Approach for High Recovery and Purity Isolation of Plasma Cells from Whole Blood

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INTRODUCTION

Characterization of Plasma Cells in Bone Marrow for different cytogenetic abnormalities is a common genetic test to diagnose and monitor cell malignant disease such as multiple myeloma (MM). Plasma cells in MM patients varied in proportion from <1% up to 100%. For the less invasive sample collection, circulating plasma cells (CPCs) in peripheral blood have emerged as an important prognostic marker in patients with MM, even though CPC burden in peripheral blood is reported to be >100-fold lower than in bone marrow.

In the past two years with the breakout of Covid-19 plasma cells in peripheral blood have also been recognized as a marker for disease resolution. It is critical to be able to enrich and isolate rare plasma cells from blood with high purity and recovery to enable sensitive detection of MM progression and SARS-CoV-2 viral infection. FACS or MACS enrichment of plasma cells are widely used to enhance CPC detection sensitivity and specificity, however the major limitation is the time-consuming sample preparation steps and the considerable loss of cells during the process.

Here we present an easy and automated method to isolate CD138+ plasma cells from peripheral blood without RBC lysis and density gradient centrifugation. The method is developed on a novel designed cell separation system, MARS® magnetic cell separator. In the pilot study, U266 cells were spiked into peripheral blood from 0.03% to 5.0% of white blood cells frequency range. We tested two series of magnetic beads and with both spiked cells were isolated at 1mL/min flow rate followed by automated rinsing and releasing on MARS®. Various dilution factors and times of purification were tested to achieve high purity at very low frequency. Purity and average 80% recovery of the plasma cells were obtained.

METHODS

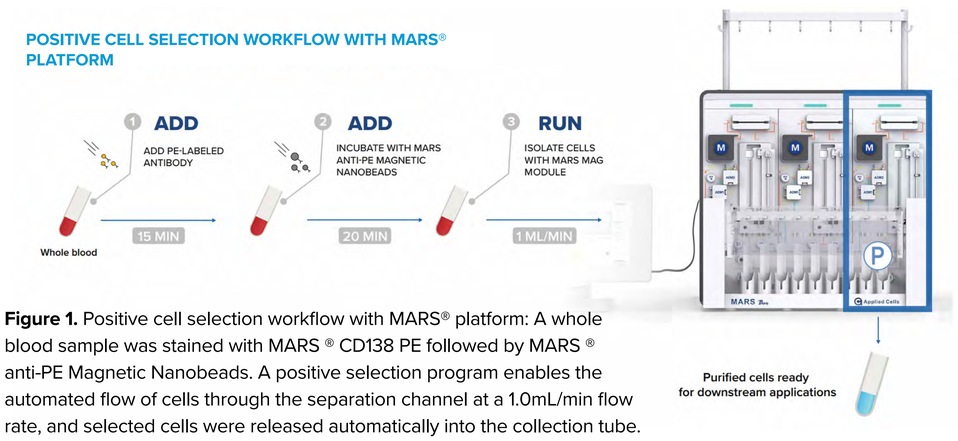

Figure 1. Positive cell selection workflow with MARS® platform: A whole blood sample was stained with MARS ® CD138 PE followed by MARS ® anti-PE Magnetic Nanobeads. A positive selection program enables the automated flow of cells through the separation channel at a 1.0mL/min flow rate, and selected cells were released automatically into the collection tube.

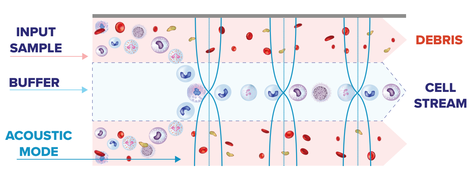

Figure 2. Negative cell selection workflow with MARS® platform combines acoustic cell wash followed by magnetic cell separation (Fig. 1) for negative isolation of untouched target cells. In the first step, the acoustic modules isolate cells from lysed whole blood by separating cellular debris and viable cells in a microfluidic channel at flow rate of 0.8mL/min.

RESULTS

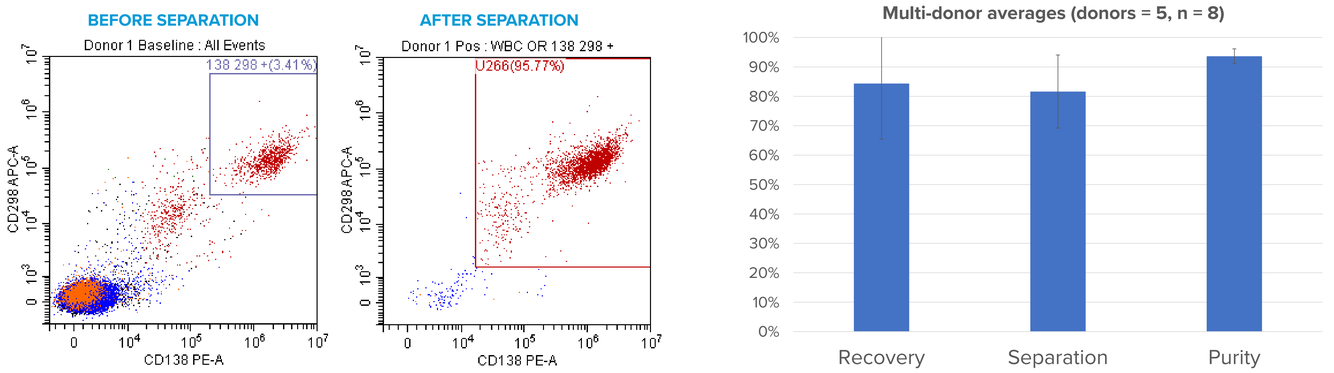

Figure 3. U266 cell line cells were pre-stained with CD298 APC and spiked in healthy donor's peripheral whole blood at the frequency of 3-5% of all white blood cells. After staining with MARS MAG-LINE reagents, blood was diluted at a 1:10 ratio with MAG BUFFER. Separation results from 5 donors and multiple replicates show an average of 94% purity and 85% recovery of CD138+ cells after separation.

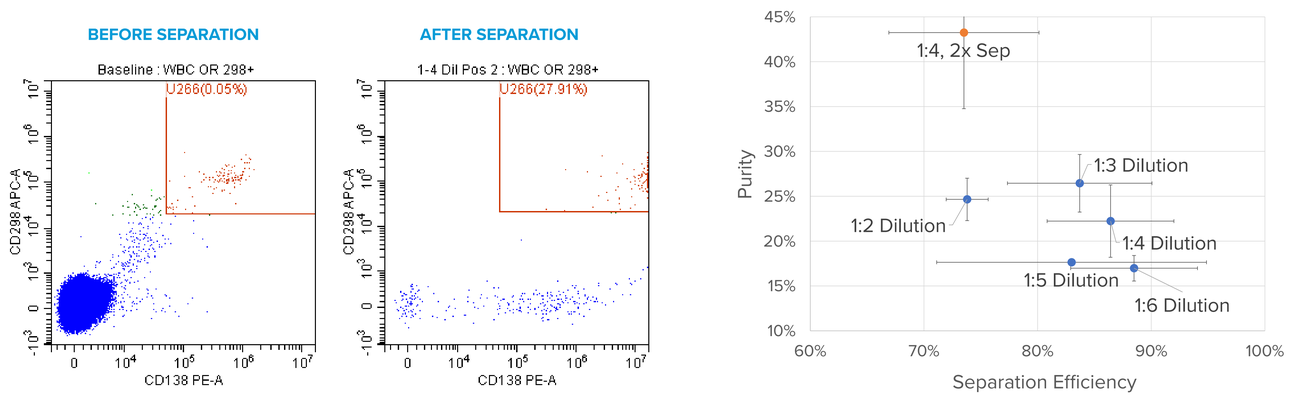

Figure 4. U266 cell line cells were pre-stained with CD298 APC and spiked in healthy donor's whole blood at the frequency of 0.05% of all white blood cells. Various dilution ratios were compared by assessing the purity of isolated cells and separation efficiency. 1:3 dilution showed higher purity and all dilution ratios higher than 2 got >80% separation efficiency. The second run of positively isolated cells did improve purity.

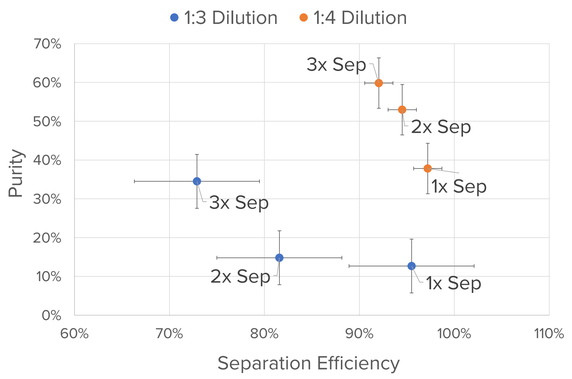

Figure 5. U266 cell line cells were pre-stained with CD298 APC and spiked in healthy donor's whole blood at the frequency of 0.03% of all white blood cells. Serial separation was applied, and increased purity was obtained when positively selected cells were run through separation 2 times and 3 times.

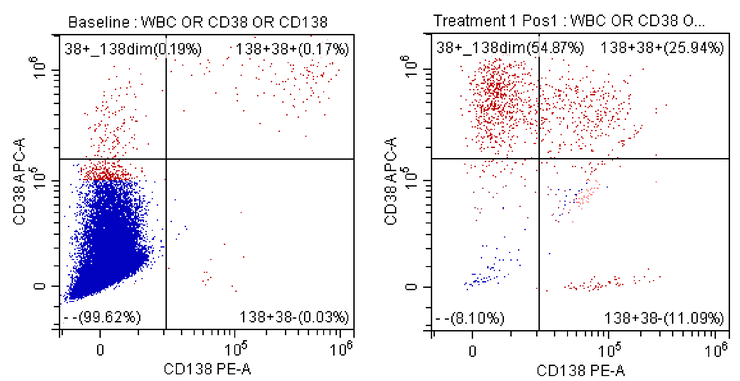

Figure 6. Results of magnetic positive selection of plasma cells from healthy donor's bone marrow. Bone marrow was diluted 1:3 with magnetic separation buffer and run through MARS MAG twice at 1mL/min flow rate each time. Plasma cells were enriched from 0.39% to 91.9%.

SUMMARY

- MARS® magnetic separation technology isolates plasma cells from whole blood and bone marrow without RBC lysis and gradient centrifugation.
- MARS® Bar system isolates plasma cells at a large range of frequency with high recovery and high purity at a high flow rate. It additionally allows automated serial separations to achieve improved purity
- MARS® platform can combine magnetic and acoustic cell separation modules offering an innovative integrated workflow for isolating untouched target cells.

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