Peripheral Blood Mononuclear Cell (PBMC) Isolation using Cell Fractionation Filters

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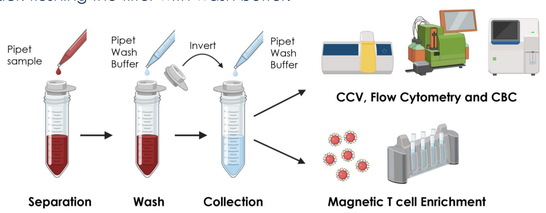
Abstract

Leukapheresis collections, the standard starting material for CAR-T cell therapies, provide sufficient cell numbers but require specialized equipment and long collection times, increasing the overall manufacturing costs and timelines. In contrast, isolating T cells from whole blood offers the potential of a safer and more cost-effective cell procurement, enhancing the overall patient experience. However, whole blood contains 10 to 100 times more red blood cells (RBCs) and platelets than leukapheresis material. These cell impurities interfere with standard T cell labeling and enrichment operations and should be removed via a peripheral blood mononuclear cell (PBMC) isolation step prior to T cell enrichment. Traditional PBMC isolation methods, such as manual or automated Ficoll gradient separation, often result in low recovery, highlighting the need for improved techniques to make whole blood a viable starting material for CAR-T manufacturing.

In this study, we explored the use of cell fractionation filters with pore sizes ranging from 5 to 9 µm, as a novel method for PBMC isolation. These filters separate the larger white blood cells (WBCs) from smaller RBCs and platelets via size exclusion. Using whole blood from two healthy donors, we identified the optimal filter pore size for RBC and WBC separation, achieving 80% CD3+ T cell recovery—a 2-fold improvement over Ficoll gradient separation. We also demonstrated >99% RBC and platelet depletion and maintained high cell viability (>94%) in the enriched PBMCs. Additionally, we assessed T cell purity and recovery after magnetic enrichment, both with and without prior PBMC enrichment. Finally, we proposed a closed-system with the cell fractionation filters. This workflow facilitates the rapid and scalable production of CAR-T therapies directly from whole blood, improving their accessibility and affordability.

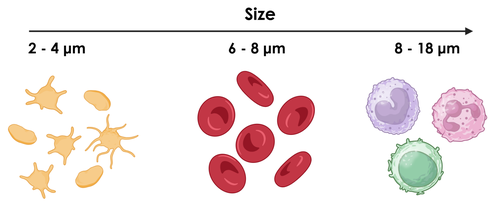
Materials and Methods

Whole blood from 3 donors was diluted 10-20X in DPBS/EDTA wash buffer with 1% Human Serum Albumin and pipetted gently onto 70% ethanol pre-treated CELLNETTA Filters, followed by a wash with additional buffer. Post wash, the filter was flipped on to a new collection tube and the retained PBMCs were collected by back-flushing the filter with wash buffer.



The retained cells were analyzed for TBNK by flow cytometry and CBC. T cells from 2-3 mL of whole blood or isolated PBMCs from 2 donors were incubated with magnetic labeling beads followed by magnetic separation to yield enriched T cells. The T cells were then analyzed for CCV and CD3+ Purity to calculate the T cell recovery.

Introduction



Approximately 40% of the volume of whole blood consists of RBCs, which significantly outnumber the PBMCs. The size difference between RBCs and PBMCs can be exploited to isolate PBMCs from whole blood. However, achieving precise separation of cells with a few micron size difference requires a precise control of the filter pore size. Here, we demonstrate efficient, gentle and robust isolation of PBMCs from whole blood using high-precision cell filters as a pre-processing step towards T cell enrichment.

5 µm Pore Size is Optimal for PBMC and CD3+ Target Cell Recovery

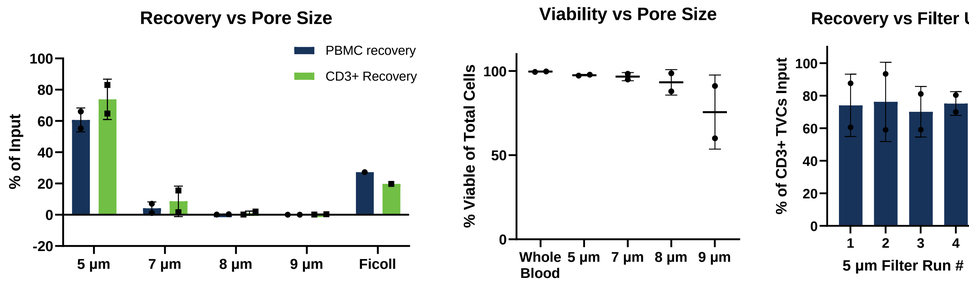


Figure 1: PBMC Isolation using different filter pore sizes. a. Total Viable Cell (TVC) and CD3+ TVC recovery from filters having 5 um , 7 um, 8 um and 9 um pore size compared with automated ficoll gradient separation. b. Viability of isolated PBMCs from filters with different pore sizes and c. CD3+ TVC recovery from repeated batches processed on a single 5 um filter. Data represented as mean ± SD of two donors.

Complete RBC and Platelet Depletion is Observed in Purified PBMCs

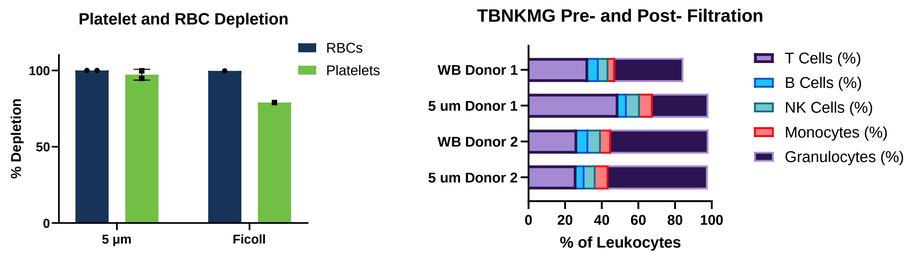


Figure 2: Composition of isolated PBMCs. a. RBC and platelet depletion in retained PBMCs compared to automated ficoll gradient separation. Data represented as mean ± SD of two donors. b. The TBNKMG composition of PBMCs in starting whole blood and enriched PBMCs from 2 donors.

T Cell Enrichment is More Efficient post PBMC Isolation

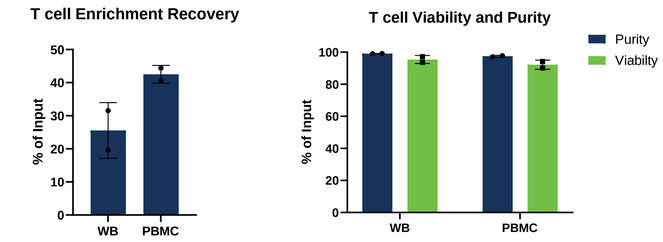
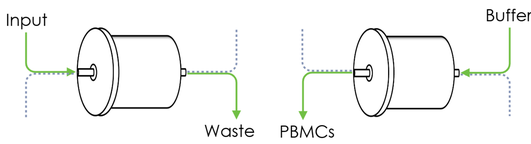


Figure 3: T cell enrichment from isolated PBMCs. a. CD3+ TVC recovery and b. CD3+ purity and viability of T cells enriched from whole blood and isolated PBMCs. Data represented as mean ± SD of two donors.

Closed System Processing



The filters can be incorporated into a closed system with tubing lines on either end that allow for repeated cycles of input and backflush.

The filters may be scaled up or scaled out to accommodate larger starting material volume.

For Illustrative Purposes Only

Conclusion and Future Directions

- PBMC isolation from whole blood using **size-based exclusion** was evaluated. Up **to 80% CD3+ T cell recovery** was achieved via a **simple filtration** unit operation.
- **Complete RBC and Platelet depletion** was observed in the isolated PBMCs, highlighting the **precise separation**.
- Downstream **CD3+ enrichment efficiency was enhanced** due to PBMC isolation.
- The filtration unit operation is **amenable to closed system** processing, but larger starting material volume may require scale-up of filter size.
- Size based separation using precision filters can also be used for separation of PBMCs into sub-populations or separation of larger, activated cells from non-activated cells.

CELLNETTA Filters supplied by Murata Manufacturing Co., Ltd. Examples of filters are on display at Inabata America Corporation's booth (#1752).
Graphs and statistical analysis performed on Graphpad Prism. Illustrations created using Biorender.

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