Applied Cells

High Performance Large Scale T Cell Isolation with MARS®

Abstract

Efficient T cell isolation with high recovery and high purity is important for academic and clinical research. Using our newly developed MARS® system (multi-physics automated reconfigurable separation) we demonstrate an automated process to isolate T cells within a short time. With MARS® Bar, we achieved greater than 95% purity of T cells isolated from a blood sample with a recovery higher than 90%. Furthermore, separated T cells were shown to have preserved activation and proliferation, necessary for further functional studies.

INTRODUCTION

T cells play a crucial role in the establishment and maintenance of immune responses, homeostasis, and memory. The advancement of T cell knowledge through T cell study led to the development of immune cell-based cancer treatment.[1] Adoptive cell therapy (ACT) with gene-modified T cells are promising strategies to modify the immune system to recognize tumor cells and carry out an anti-tumor effector function.[2]

Human T cells can be isolated, cultured, expanded, and manipulated for research investigations. An efficient way to isolate T cells is therefore important for basic and clinical research applications Automated MARS® Bar (multi-physics automated reconfigurable separation) parallel cell separation system incorporates Applied Cells' proprietary immuno-magnetic separation technology. The cell separation modules separate cells bound to magnetic beads from cells unbound to magnetic beads in flow channels without the need for dedicated columns and enable purification and enrichment of up to 3 samples in parallel. The system was designed to provide a fully automated, walk-away workflow.

With the MARS® Bar system, we have achieved greater than 95% T cells purity and above 90% CD3 recovery from whole blood apheresis product within 45 min. The entire process did not affect T cell viability and functionality.

OBJECTIVE

The goal of the experiment was to enrich human CD3+ T cells as a starting material for T cell therapy development and production in a large scale with:

- High purity and high recovery
- High throughput
- Ease of use and within a short time

Additionally, we aimed at testing the preserved T cell activation and proliferation.

METHODS

One blood product from Leukoreduction System Chambers was incubated with CD3 binding magnetic beads, diluted with one volume of PBS, and then followed by MARS® magnetic separation.

RESULTS

MARS® Bar system was designed to minimize time and generate fully automated workflows for efficient large-scale cell separation.

Before separation, the blood cell sample required minimal processing, which involved 30 min incubation of the blood sample with magnetic beads (**Figure 1**). After the incubation, cells were separated on one of three MARS Bar magnetic separation modules at a high rate of 1 mL/min.

The recovery and purity of separated cells were analyzed by staining cells with CD3, CD19 and CD45 antibodies in the input sample, positive and negative selection fractions. The analysis revealed that more than 90% of CD3 cells were recovered with 95% purity (**Figure 2**). The recovery was calculated based on CD3 events recorded in 50uL of flow cytometry sample and the volume collected before and after magnetic separation.

To detect T cell activation hallmarks, CD3/CD28 activation was followed. Upregulated CD25 expression on the CD3/CD28-activated T cells, but not the unstimulated control, was detected at day 3 post-activation (**Figure 3A**). Additionally, the CD3/CD28 activated T cells, but not the unstimulated control, characterized with increased diameter (**Figure 3B**, data represents samples analyzed on day 7). Finally, active proliferation was detected only for the CD3/CD28 activated T cells, but not the unstimulated control (**Figure 3C**).

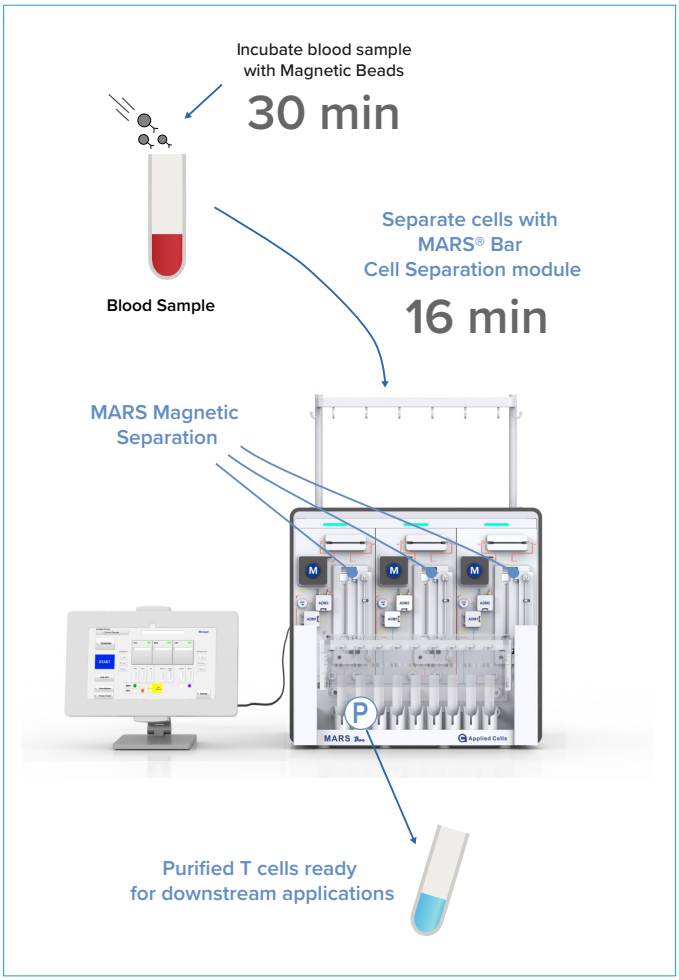

Figure 1. MARS® Bar T cells magnetic separation workflow.

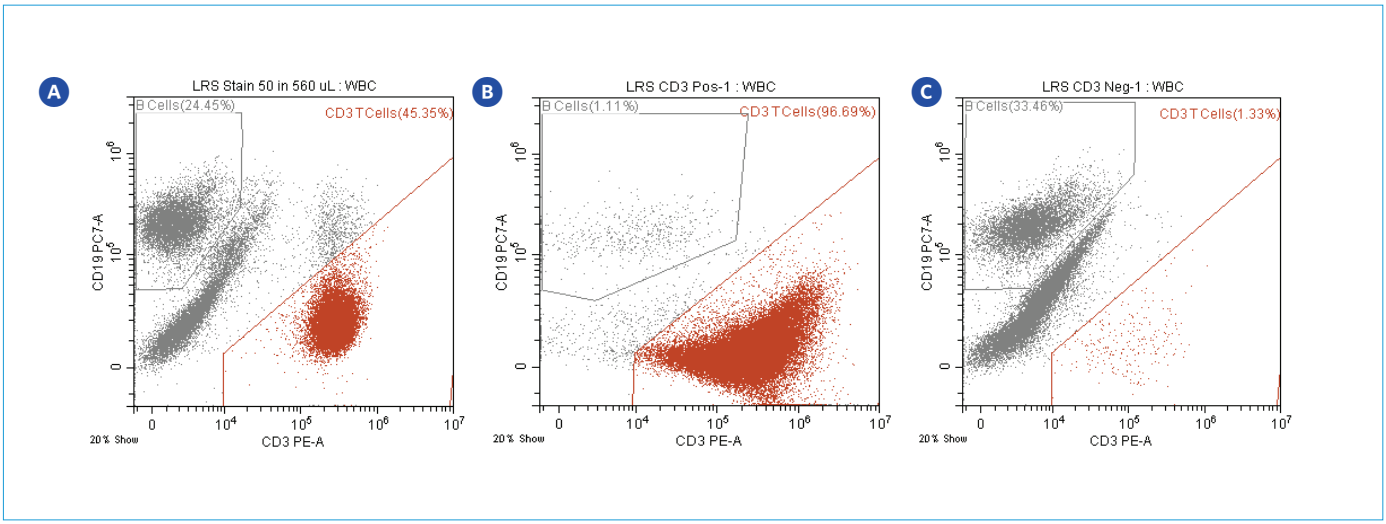

Figure 2. Analysis of the positive selection fraction and negative selection revealed above 95% CD3 purity and above 90% CD3 recovery by MARS Bar magnetic selection. **(A)** CD3 and CD19 populations in CD45+ cells before T cell separation; **(B)** CD3 and CD19 populations in CD45+ cells in the Positive fraction, and **(C)** CD3 and CD19 populations in CD45+ cells in the Negative fraction.

T cell phenotypes and their memory subsets were evaluated 7 days after activation. **Figure 4** shows that cell viability was above 90% and over 98% of the cells were T cells with CD3 expression. More than 90% of the T cells constituted CD4 T helper and CD8 cytotoxicity T cell subsets. Finally, central memory T cells were observed at the level of 80% of the T cell population, as evaluated by CD45RA negative and CD62L positive.

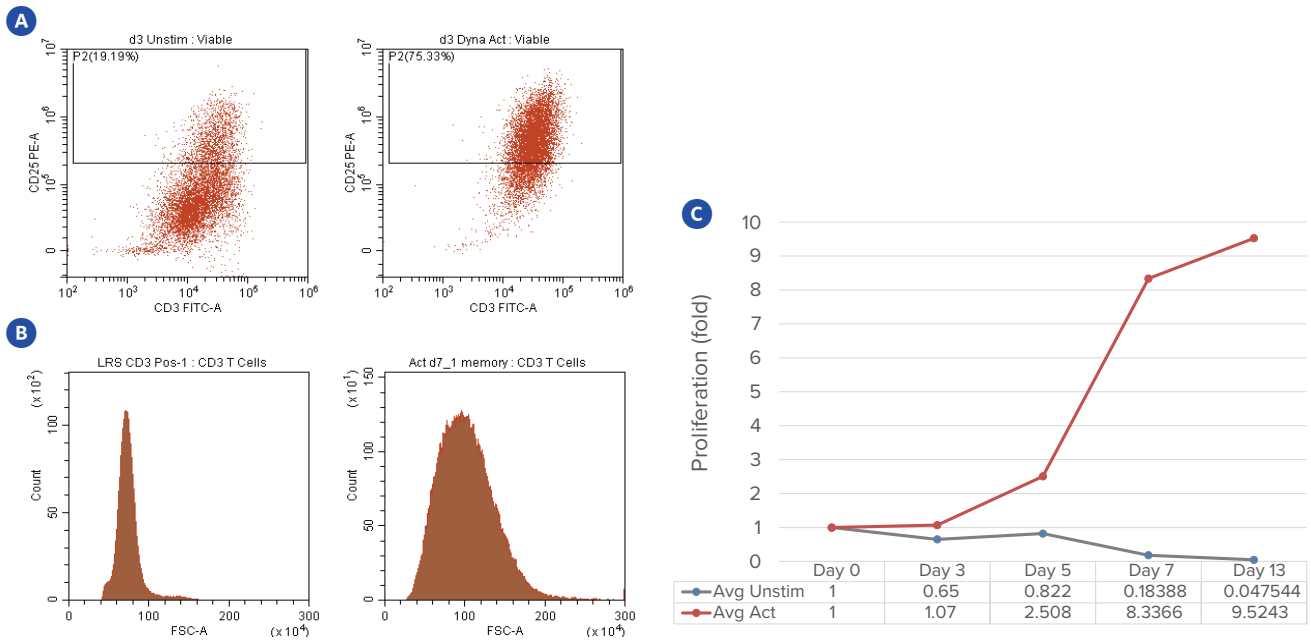

Figure 3. T cell activation hallmarks were detected in a CD3/CD28-activation dependent manner. (A) Upregulated CD25 expression on the CD3/CD28-activated T cells, but not the unstimulated control, was detected at Day 3 post-activation. (B) Increased cell diameter on the CD3/CD28-activated T cells, but not the unstimulated control, was detected. Representative data shown with Day 7 samples. (C) Active proliferation was detected for the CD3/CD28-activated T cells, but not the unstimulated control.

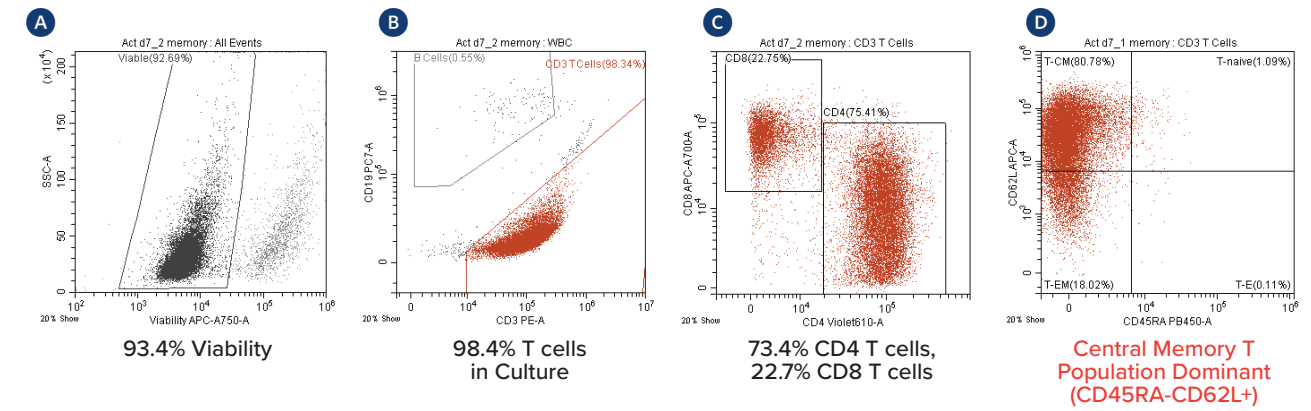

Figure 4. T cell phenotypes and their memory subsets were evaluated at day 7 post-activation. **(A)** Cell viability was above 90%, **(B)** 98% of the cells were T cell with CD3 expression, **(C)** Above 90% of the T cells was composed of CD4 T helper and CD8 Cytotoxicity T cell subsets, **(D)** Central memory T cells were observed at 80% of the T cell population, evaluated by CD45RA negative and CD62L positive.

DISCUSSION

Purified T cells can be used in many downstream applications, such as T cell culture, T cell functional assay, molecular study, immunotherapy, etc. As key players in anti-tumor responses, the therapeutic applications are broadly adopted by immuno-oncology research.

MARS Bar system is an automated solution to rapidly isolate T cells with high purity and recovery. Using one of three modules within the MARS Bar system, we have purified 120 million CD3 T cells from a 260 million total white blood cells sample in approximately 45 min, with a 16+ million cells/min separation rate. The workflow provides flexibility to isolate CD3+ T cells and subsets of T cells from peripheral blood in a short time.

The isolated T cells can be further used in cultures with the regular procedure since MARS-mediated cell separation causes no harm to cell viability and functionality. In addition, MARS® Bar can be placed in a biosafety cabinet to perform sterile cell sorting to isolate sterile T cells for expansion or other downstream applications.

CONCLUSIONS

MARS systems are automatic sample preparation platforms incorporating multiple separation technologies. Using our proprietary immuno-magnetic separation technology, we can enrich ~500 million cells per single run in a short time.

MARS Bar allows for the separation of 150-200 million target cells on each of the three magnetic modules with a processing rate of 1 - 20 million cells/min. The system offers a flexible configuration for a variety of DOE needs.

MARS® Cell Separation System

REFERENCES

1. Kumar, B.V., T.J. Connors, and D.L. Farber, Human T Cell Development, Localization, and Function throughout Life. *Immunity,* 2018. 48(2): p. 202-213.
2. M. W. Rohaan, S. Wilgenhof, and J. B. A. G. Haanen corresponding, Adoptive cellular therapies: the current landscape. *Virchows Arch.* 2019; 474(4): 449–461.

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