Column-Free CD14+ Monocyte Isolation using 50nm Superparamagnetic Beads on MARS® Bar

INTRODUCTION

Dendritic cells are professional antigen presenting cells and they play a key role in the regulation of immune responses. Dendritic cells are widely used as vaccine and drugs for cancer treatment in large number of trials. Macrophages are essential innate immune cells and when activated, macrophages can mediate the phagocytosis of dangerous cells or materials and participate in effective tissue regeneration. Thus, Ex vivo-generated macrophages have been used in clinical trials as cell-based therapies. Both dendritic cells and microphages can be generated from monocytes in peripheral blood. To allow robust, reproducible and simplified operation on monocyte isolation, new technology and products are demanded

Conventional micron-sized beads are known to have low efficiency and require de-beads step; however, 50nm superparamagnetic particles have larger surface area and don't have to be removed from cells for downstream applications. Commercially available 50nm magnetic beads have been used in clinical applications. However, the current commercially available 50nm magnetic beads must be used with columns for efficient separation. Here we present the CD14+ monocyte isolation using Applied Cells Ingenuity™ 50nm beads with our propriety column-free technology on a closed automated system MARS® Bar.

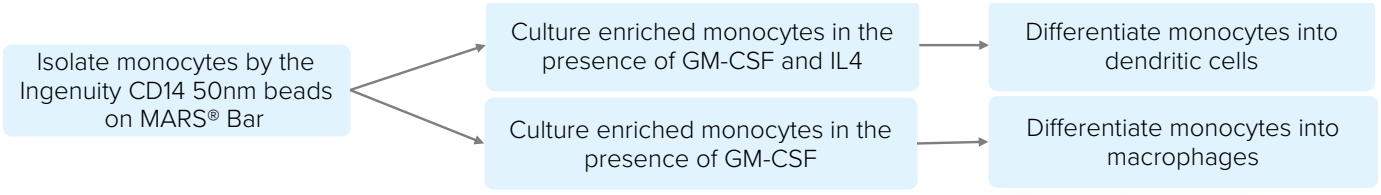

Figure 1. Workflow of monocyte positive selection and subsequent differentiation.

Applied Cells Ingenuity™ Reagents

- 50nm size superparamagnetic beads
- High safety, biodegradable particles
- High stability, excellent separation efficiency of target cells
- Provide RUO and GMP grade products

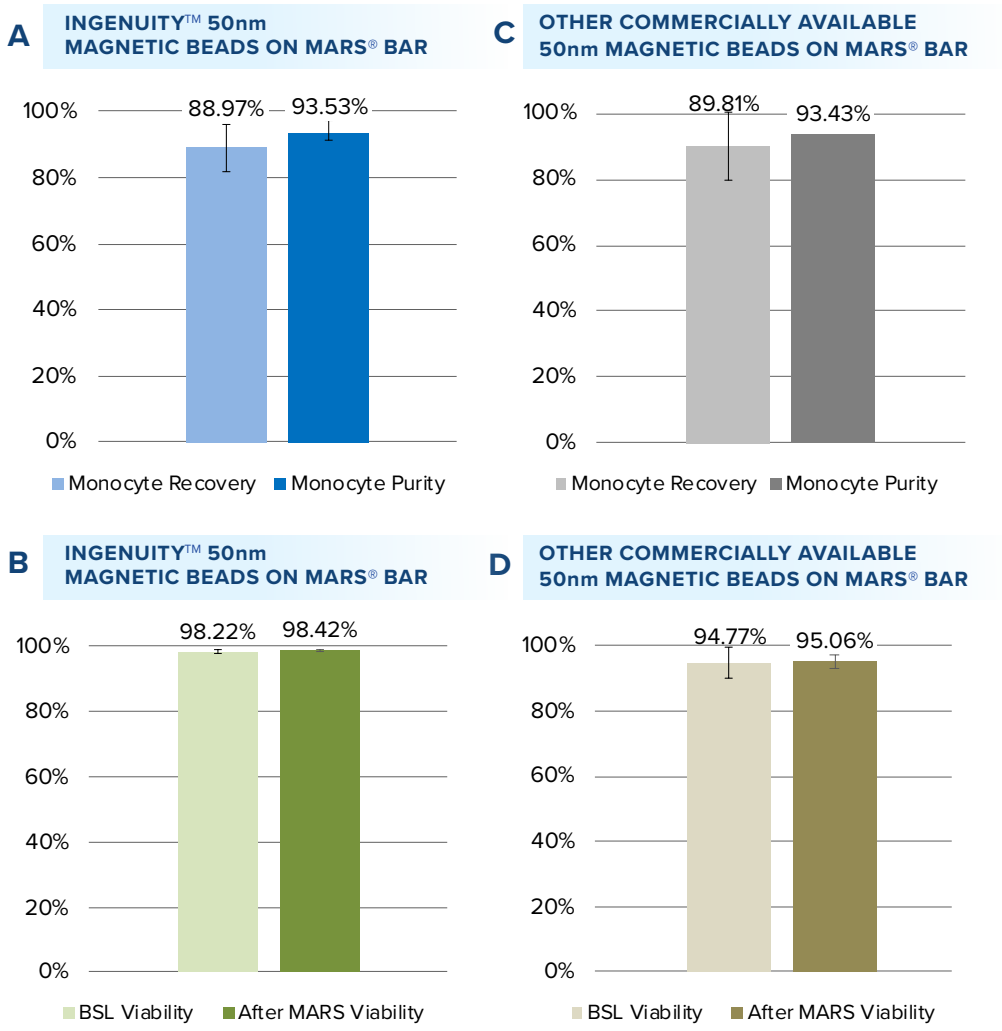

Figure 2. Monocyte isolation from peripheral blood mononuclear cells (PBMC) was performed using the MARS® Bar platform. Monocytes were isolated via positive selection with no notable change in viability. (A) Results of monocyte isolation using Ingenuity™ 50nm CD14 direct conjugate beads: on average, 88.97% of monocytes were recovered with a mean monocyte purity of 93.53%, starting from 14.68% average purity; (B) Viability analysis: pre and post Ingenuity™ 50nm CD14 beads positive selection showed no significant effect (P<0.5); n = 4 PBMC samples. (C) Results of monocyte isolation using other commercially available 50nm CD14 beads on the MARS® Bar platform: An average of 89.81% of monocytes were recovered with a mean monocyte purity of 93.43%, starting from 14.53% average purity; n = 4 PBMC samples; (D) Viability assessment: pre and post commercially available 50nm CD14 beads positive selection revealed no significant effect (P<0.5).

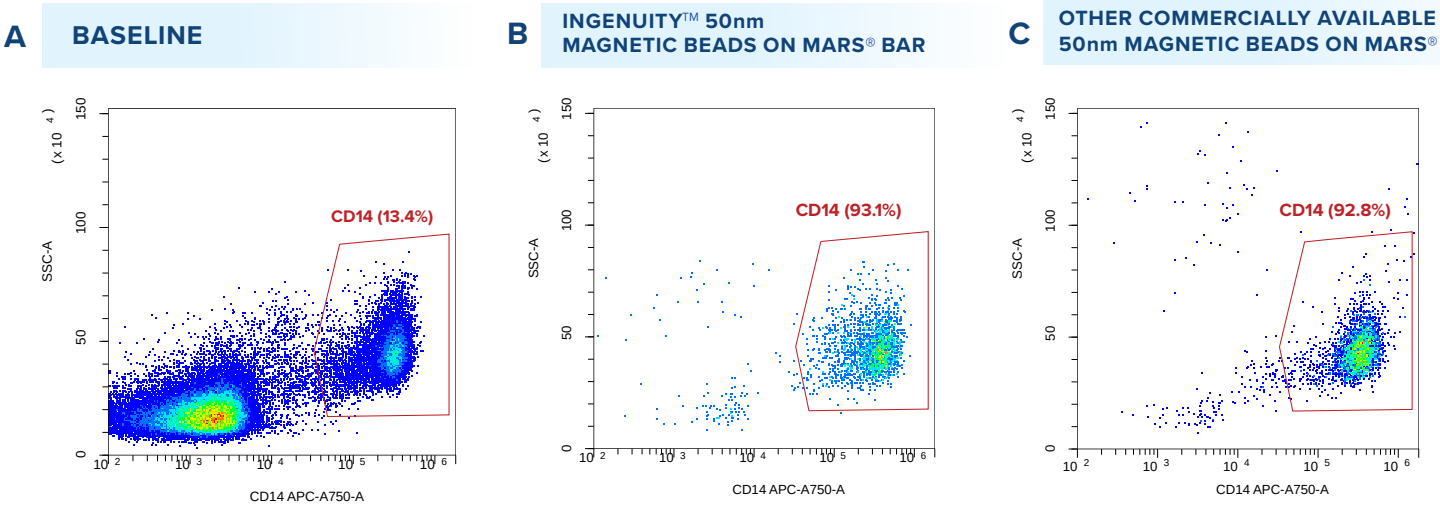

Figure 3. Purity of human monocytes isolated using MARS® Bar Platform. Dot plots show (A) PBMC before separation, (B) monocytes enriched with Ingenuity™ 50nm beads, and (C) monocytes enriched with alternative commercially available 50nm beads. Figures 3(B) and 3(C) correspond to the median performance of the data presented in Figures 2(A) and 2(C), respectively.

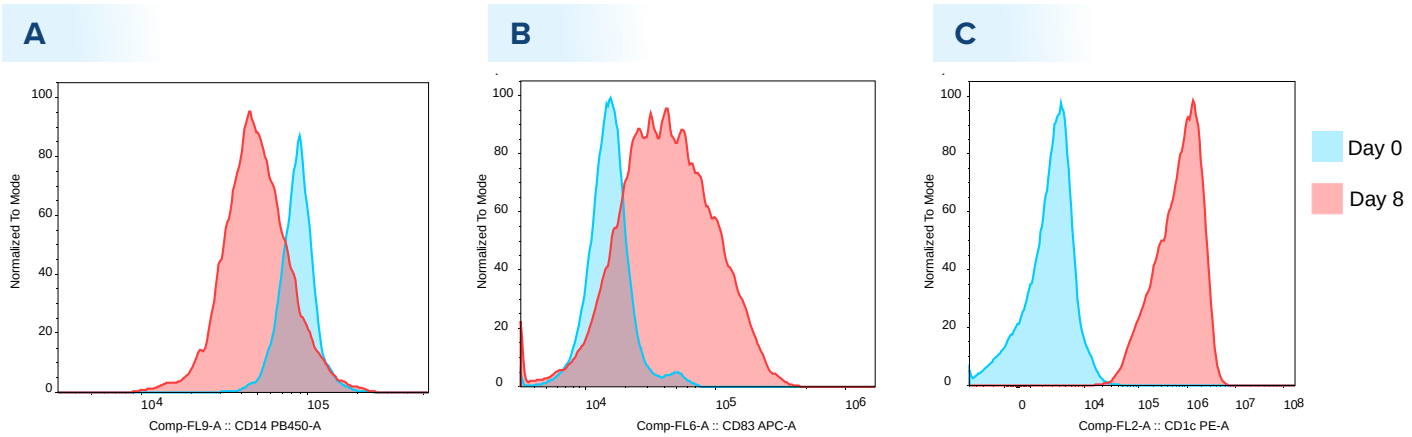

Figure 4. Ingenuity™ 50nm CD14 beads enriched monocytes were cultured to derive dendritic cells, demonstrating characteristic phenotypic features typical of dendritic cells. Monocytes were isolated and cultured for 8 days in the presence of GM-CSF and IL4. By day 8 of differentiation, the monocytes isolated with Ingenuity™ 50nm beads using MARS® Bar exhibited down-regulated expression of CD14 (A), and up-regulated expression of dendritic cell markers CD83 (B) and CD1c (C), when compared to day 0 levels.

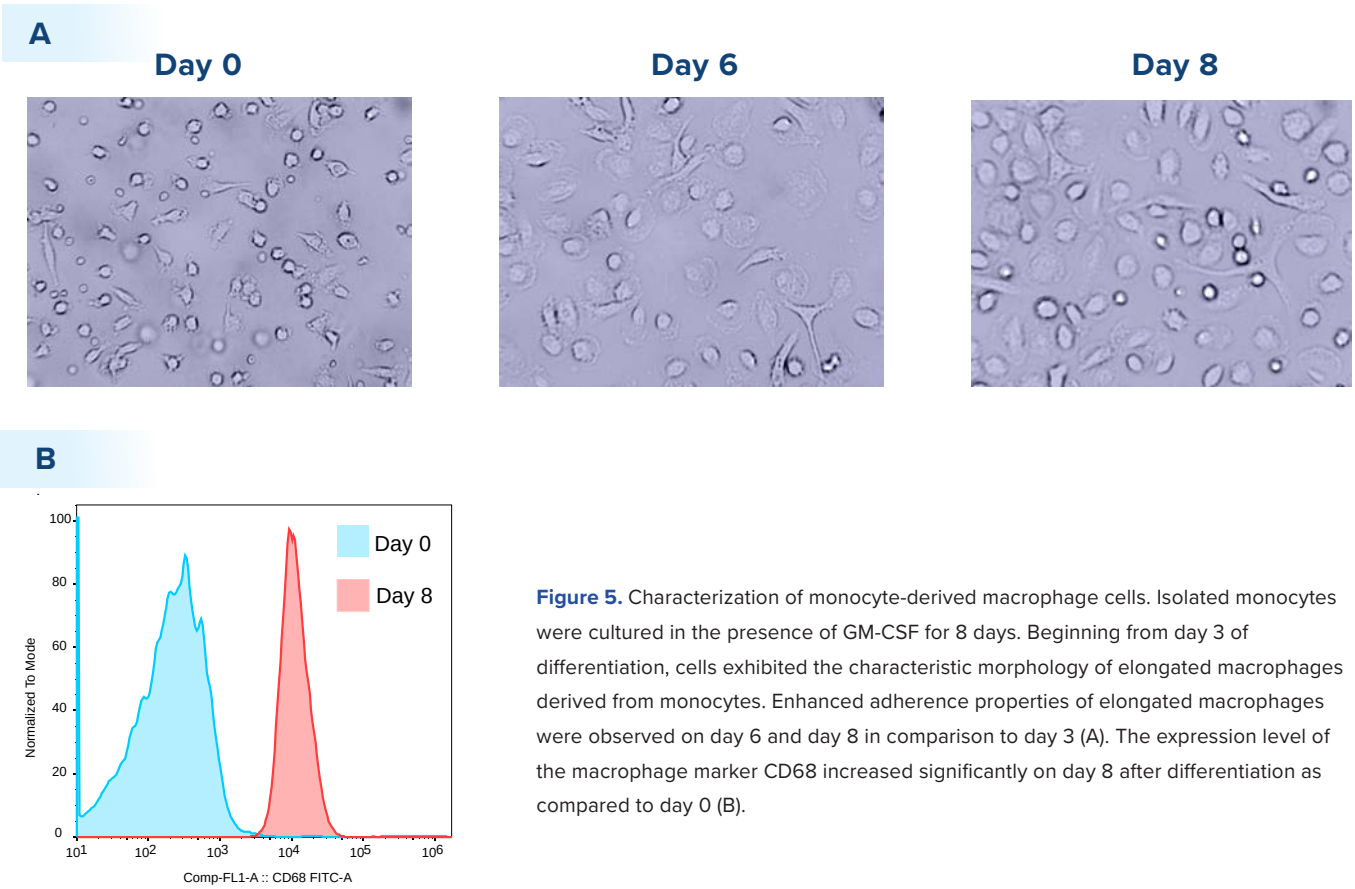

Figure 5. Characterization of monocyte-derived macrophage cells. Isolated monocytes were cultured in the presence of GM-CSF for 8 days. Beginning from day 3 of differentiation, cells exhibited the characteristic morphology of elongated macrophages derived from monocytes. Enhanced adherence properties of elongated macrophages were observed on day 6 and day 8 in comparison to day 3 (A). The expression level of the macrophage marker CD68 increased significantly on day 8 after differentiation as compared to day 0 (B).

About MARS® Bar

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MARS® Bar Cell Separation Platform

The MARS® Bar Magnetic Separation Platform is a closed and automated isolation for cell therapy development and manufacturing. Together with fit-for-purpose consumables, it delivers high cell purity, recovery, and viability.

Designed to be used with magnetic beads, MARS® Bar Magnetic Separation Platform uses column-free technology performs in-flow separation at high flow rate to achieve exceptional cell recovery, purity, a wide range of reaction volumes while maintaining cell viability. Together with process flexibility, sterile single-use consumables, and software with 21 CFR Part 11 compliance, this system is designed to help you easily scale from development to clinical and commercial manufacturing.

FLEXIBLE PROTOCOLS
BROAD RANGE OF VOLUMES AND SAMPLE TYPES
FAST AND EASY PROTOCOL OPTIMIZATION

FULLY ENCLOSED
AUTOMATED
ECONOMICAL

Discovery — RUO

Development — RUO

Manufacturing — GMP

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