Application note Vol.6

Under Development

Specification and appearance are subject to change without notice

CELLNETTA MZM1 SERIES

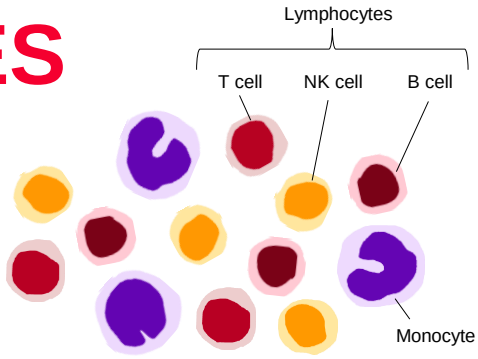

Illustration of PBMC

Monocyte removal for the fractionation of lymphocytes from PBMCs

Background

There is extensive research being conducted on regenerative medicine products that use human blood and tissue as raw materials for drugs. The specific cells used as these raw materials for drugs should not be contaminated with other cells. Therefore, a highly accurate method is required to extract the desired cells from the original blood or tissue. Magnetic bead separation is used as a method for separating cells, but the reaction between cells and antibodies, as well as the process of washing the antibodies after separation, makes it a time-consuming process. Therefore, we propose CELLNETTA, which can help speed up the process. Cells of different sizes can be easily sorted by simply pouring the cell suspension through CELLNETTA.

Here, we will introduce a case study on the use of CELLNETTA for the removal of monocytes for the fractionation of lymphocytes from human PBMCs (peripheral blood mononuclear cells) , conducted by Dr. Norihisa Mikami of the Research and Development Department of RegCell Co., Ltd.

Implementation method

(1) Use buffer*1 to adjust the cell count of the human PBMCs to 5×10^6 cells/mL.
(2) Apply hydrophilic treatment to the CELLNETTA. *2
(3) Pour 1 ml of the cell suspension prepared in (1) through CELLNETTA.
(4) Ensure that the fluid flows through, then pour 5 mL of buffer.
(5) Use magnetic beads to further purify the cell suspension that has flowed through CELLNETTA, then analyze using flow cytometry*3.

*1 How to prepare the buffer (for 500 ml sample)

D-PBS	489 mL
FBS	10 mL (final concentration of 2%)
0.5 mol/L EDTA solution	1 mL (final concentration of 1 mM)

*2 For more information, please refer to the “Hydrophilic Treatment Manual” in the CELLNETTA User Guide.

*3 For magnetic bead purification and flow cytometry procedures, please refer to the manufacturer's operating procedures.

Summary of results

Human PBMCs contain a large number of lymphocytes and monocytes (Figure 1). Because lymphocytes have a smaller cell size than monocytes, CELLNETTA was used in an attempt to sort them by size. Processing human PBMCs using a 7-µm mesh CELLNETTA showed a decrease in monocytes in the flow-through fraction (Figure 1). Next, in order to adapt to the preparation of T cells to be used as raw materials for regenerative medicine products, CD4-positive T cells were purified with magnetic beads after monocyte removal using CELLNETTA or anti-CD14 antibody labeled magnetic beads. As a result, both CELLNETTA and CD14 negative selection suppressed the monocyte contamination observed in untreated human PBMCs after CD4 purification (Figure 2).

These results indicate that CELLNETTA can be used to remove monocytes and to fractionate lymphocytes from human PBMCs. The simple and quick operation of pouring human PBMCs through CELLNETTA can save time when compared to conventional methods. For this reason, it is expected to contribute to reducing the manufacturing costs of regenerative medicine products.

Detailed results

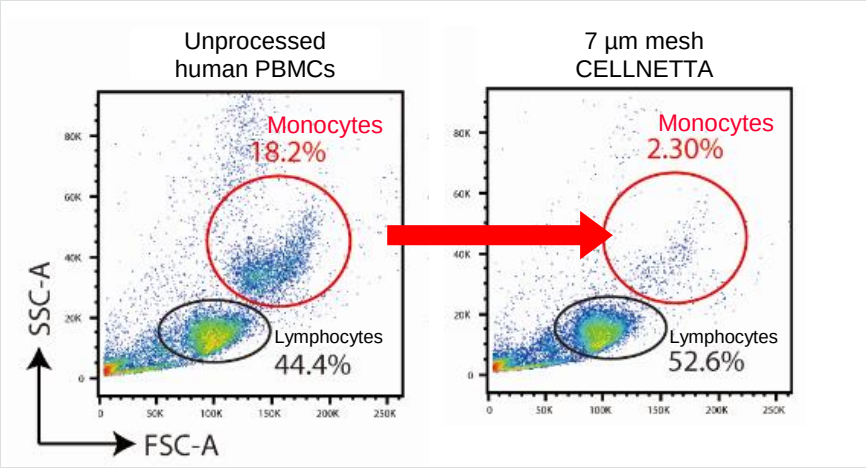

Figure 1 Results of flow cytometry analysis
: use of CELLNETTA reduced the percentage of Monocytes.

Application note Vol.6

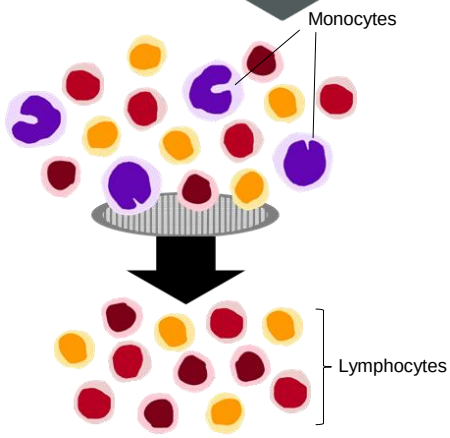

Illustration of lymphocyte fractionation from human PBMCs

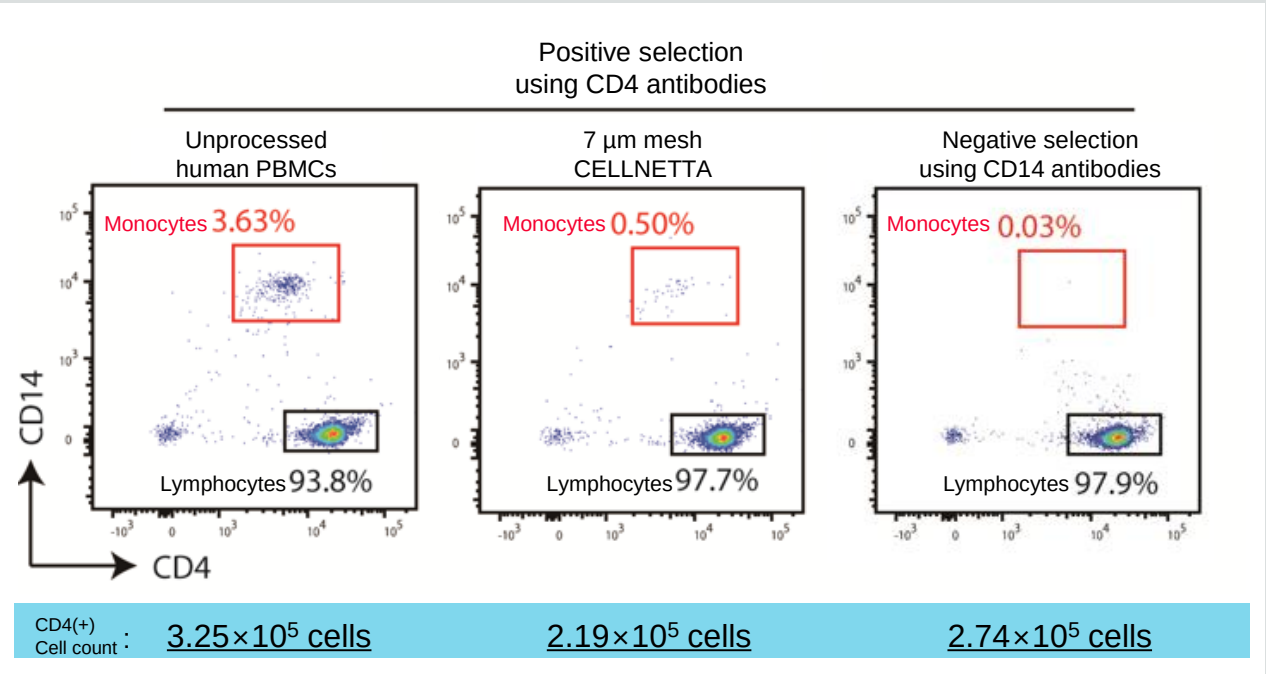

Figure 2 Results of magnetic bead purification and flow cytometry analysis of the number of CD4-positive cells
: CD4-positive cells can be recovered using CELLNETTA or CD14 negative selection.

Product used in this application note

Pore size	Gamma Irradiation	Product number (P/N)
7 µm	Gamma Irradiated	MZM1B007B50G

Notes

- This product is not a medical device.
- This product is a sample for evaluation purpose.
- Please do not ship out your completed product with the sample.
- We shall not be liable for any claims on the sample in case it is shipped out to the market.

muRata
INNOVATOR IN ELECTRONICS

Murata Manufacturing Co., Ltd.

CELLNETTA Website