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Abstract No. 753

A method for rapid isolation of highly purified monocytes using fully automated negative cell selection

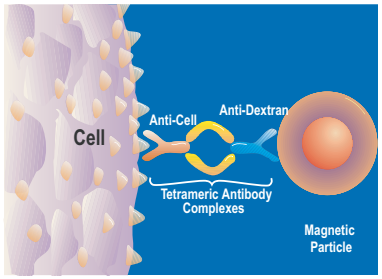
Ning Yuan[1], Benoit G. Guilbault[1], Graeme T. Milton[1], Jodie Fadum[1], Jackie E. Damen[1], Allen C. Eaves[2], Terry E. Thomas[1], Bert W. Wognum[1]. [1]StemCell Technologies Inc., 570 W. 7th Avenue, Vancouver, BC, V5Z 1B3, Canada, [2]Terry Fox Laboratory, 675 W. 10th Avenue, Vancouver, BC, V5Z 1L3, Canada.

Summary

Monocytes participate in both innate and acquired immune responses. They are responsible for phagocytosis of foreign substance in the body and are capable of killing infected host cells through antibody dependent cytotoxicity. Moreover, monocytes are able to differentiate to macrophages and dendritic cells (DC), serving as scavenger and antigen presenting cells. The preparation of highly purified monocytes for experimentation has traditionally been difficult, requiring multiple steps and many hours of work. In addition, purity and recovery may be low, and purified monocytes are often activated by cell labeling and cross-linking to magnetic particles. We have developed a rapid negative selection method that enables the preparation of highly purified monocytes from human peripheral blood using EasySep® column-free immunomagnetic cell separation technology. We also optimized the method for use with the RoboSep® fully automated cell separator. Both the manual and fully automated separation procedures took less than 30 minutes and provided average $CD14^+CD16^-$ monocyte purity above 90% combined with average recoveries above 60%. Culture of purified monocytes in the presence of GM-CSF, IL-4 and LPS led to efficient differentiation into dendritic cells (DC) as identified by the expression of the DC markers CD1a and CD83, and the loss of the monocyte marker CD14. Incubation of the differentiated DC with allogeneic $CD4^+$ T cells induced robust $CD4^+$ T cell proliferation, which further confirmed that the isolated monocytes were fully competent to differentiate into functional DCs. We report here the successful development of a new one-step method for isolating highly purified and functional monocytes.

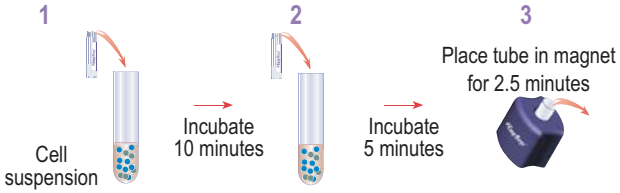
Methods

Figure 1. Schematic drawing of EasySep® magnetic labeling of human cells



TAC are comprised of two mouse IgG1 monoclonal antibodies held in tetrameric array by two rat anti-mouse IgG1 monoclonal antibody molecules. One mouse antibody recognizes the specific cell surface antigen while the other recognizes dextran on the EasySep® magnetic particle.

Figure 2. EasySep® procedure for column-free enrichment of human monocytes by negative selection



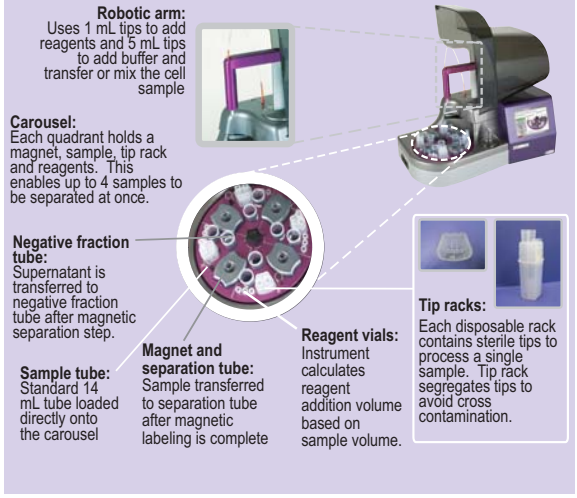
Add EasySep® selection cocktail containing tetrameric antibody complexes that target the following markers on unwanted cells: CD2, CD3, CD16, CD19, CD20, CD56, CD66b, CD123, glycophorin A.

Add EasySep® magnetic particles

Invert magnet to pour off desired fraction into a new tube. The magnetically labeled unwanted cells remain bound in the tube by the magnetic field.

Obtain purified cells in less than 30 minutes.

Figure 3. Fully automated enrichment of human monocytes using RoboSep®



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Results

Figure 4. EasySep® negative selection technology provides highly purified monocyte fractions.

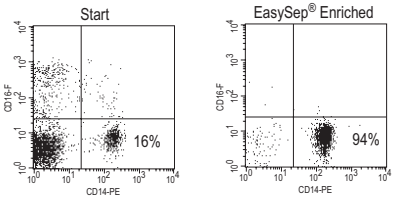
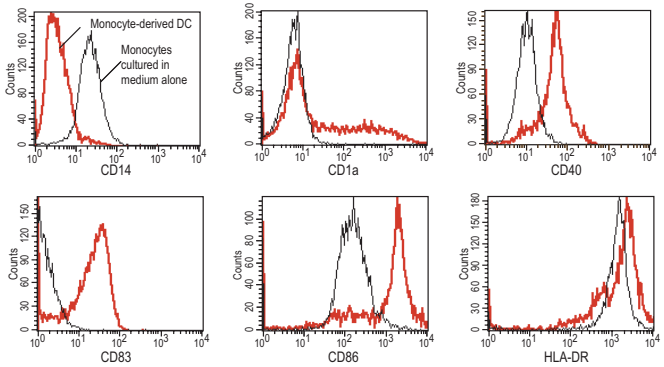


Table 1. Results obtained using EasySep® and RoboSep® technology for human $CD14^+$ $CD16^-$ monocyte enrichment from previously frozen mononuclear cells*

	%$CD14^+CD16^-$ Cells			Recovery from start	
	Start	EasySep®	RoboSep®	EasySep®	RoboSep®
AVERAGE (n=7)	19.8	90.1	90.1	63	71
SD	8.0	3.4	4.6	19	21

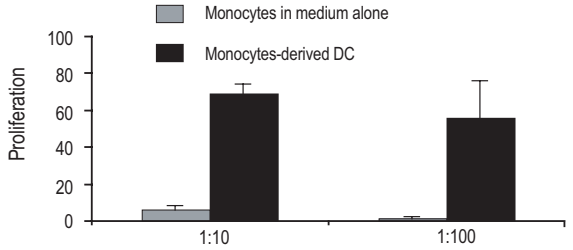
*Mononuclear cells obtained from peripheral blood Leuko Paks collected from normal donors using an apheresis machine were further processed using density centrifugation and cryopreserved in FBS containing 7.5% DMSO prior to use.

Figure 5 Monocytes isolated using EasySep® technology can be differentiated to dendritic cells.



Enriched monocytes were differentiated into DC by culturing for 7 days in the presence of GM-CSF, IL-4 and LPS (red line). Enriched monocytes were also cultured in medium alone and served as undifferentiated controls (black line). Cell surface expression of CD14, CD1a, CD40, CD83, CD86 and HLA-DR was measured by flow cytometry.

Figure 6 Dendritic cells derived from monocytes isolated using EasySep® technology are capable of inducing T cell proliferation in mixed lymphocyte reactions.



Allogeneic $CD4^+$ T cells isolated using EasySep® technology were labeled with CFSE and cultured for 7 days in serum-containing medium in the presence of monocytes cultured in medum alone or with cytokines and LPS (monocytes-derived DC) at a cell to $CD4^+$ T cell ratio of 1:10 and 1:100. Cell division was analyzed by measuring CFSE in gated viable $CD3^+$ cells using flow cytometry. Results are expressed as %cells having undergone ≥ 1 cell division event. Bars are the means of 3 independent experiments, each performed with triplicate culture with Error Bars depicting standard deviations from the mean. No $CD4^+$ T cell proliferation was detected when $CD4^+$ T cells were cultured in medium alone, whereas stimulation of $CD4^+$ T cells with anti-CD3 anti-CD28 coated beads for 7 days resulted in 94% of the cells dividing (data not shown).

Conclusions

- Functional monocytes can be isolated in single step using column-free immunomagnetic EasySep® technology.
- Highly purified cells can be obtained in less than 30 minutes.
- The method can be fully automated using the RoboSep® cell separator.