

Simple Isolation of Human Peripheral Blood Monocytes Using Density Gradients of Percoll

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Abstract

Background: Monocytes play important roles in human immune system and are precursors of macrophages in body tissues. They have responsibility for phagocytosis of foreign substances and present antigens to T lymphocytes including producing many cytokines in order to control the immune system.

Objectives: 1) To study and establish the method of human blood monocyte isolation by using Percoll density gradient as an alternative method. 2) To determine monocyte purity, viability, yields and phagocytosis function after isolation by percoll density gradient.

Methods: Acid citrate dextrose blood from 13 healthy blood donors was obtained from blood bank and the buffy coat of blood sample of each donor was used in each experiment. Two step procedure with single gradient in each step for monocytes isolation from whole blood was used. First, we used a Ficoll-Hypaque gradient (density = 1.070 g/ml) for separation of peripheral blood mononuclear cells (PBMC) and then a slight hyperosmolar percoll gradient (density = 1.064 g/ml). PBMC was counted and viability was estimated by trypan blue dry exclusion. Percentage of monocytes after the Percoll gradient was determined by CD45/CD14 staining and analysis using Flow cytometer. The functional monocytes were detected by phagocytosis of latex beads.

Results: Our study demonstrated high percentage of viability of both PBMC and monocyte determined by trypan blue exclusion (95.3 and 94.1, respectively). The average of percentage of monocytes present in the PBMC recovered from the initial Ficoll-Hypaque gradient was found to be 21.0 % monocytes. From PBMC 150×10^6 cells, using Percoll gradient, an average of 16.8×10^6 monocytes with a purity of 81.3% and a recovery of 43.1% were obtained. The functional monocyte detected by phagocytosis of latex beads was shown to be 74.8 %.

Conclusion: Percoll density gradient procedure provides highly purified human monocytes and can be done with usual reagents and equipment of average laboratory. Thus, this procedure is still attractive alternative method for resource limited settings because it is convenient, simple and cheap and can be applied for the other immunological research.

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