Original Research Article

SEPARATION AND OPTIMIZATION OF A SUCROSE DENSITY GRADIENT CENTRIFUGATION PROTOCOL FOR ISOLATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC)

Abstract:

One step centrifugation procedure used commonly for separation of blood cells is the ficoll gradient centrifugation. In this method, after centrifugation, the peripheral blood mononuclear cells (PBMCs) are located on the top of the separation fluid, whereas other blood cells erythrocytes and granulocytes sediment to the bottom. In the present study 75% of lymphocyte suspension could be separated by using a one-step density gradient centrifugation of sodium heparin blood with Sucrose. Sucrose was diluted into different concentrations using miliQ water (10%,20%,30%,40%,50%,60%,70%,80%,90%,100%,). A mL volume of diluted blood was layered on 4 mLs of each sucrose solution and centrifuged for 45 minutes at 1000 rpm. Clear separation of PBMCs could be observed in solution with 40% sucrose. The separated PBMCs were analyzed in heme analyzer which showed 75% lymphocytes, 23% monocytes and 2% of other cells.

Keywords: Sucrose; PBMCs; Gradient centrifugation; Blood

1. Introduction:

Blood is made up of plasma and various types of blood cells including PBMCs. PBMCs are small white blood cells (WBCs), varying from 7 to 8 micrometers in length. In the human body, WBCs are the only cells which can be transformed to proliferate. LYMPHOCYTES originate from lymphatic tissue which is present ~~in~~ throughout the body [1].

WBCs protect the body from diseases, invasion of foreign bodies, tumors and infections. They are used for the immunological studies directed to histocompatibility antigens and cellular immunity; cytotoxicity studies; in determining the proportion of T cells to B cells, and in separating helper T cells and Suppressor T cells [1,2].

The separation of WBCs is carried out by density gradient centrifugation [3, 4, 5]. The separated blood components can subsequently be used for their respective clinical and scientific applications and investigations. At present to separate and isolate different parts of blood, commercially available ficoll is used for centrifugation which is highly costly [6, 7, 8]. In this background, the present study was undertaken to simplify and make it cost effective process.

2. Materials and methods

The materials used in the study are as follows: 1X PBS Buffer, Sucrose, Human blood in Heparinized green top tubes, Centrifuge tubes, Centrifuge.

A non-reducing disaccharide, sucrose is composed of glucose and fructose linked by anomeric carbons. Commercially it is obtained from sugarcane plant and extensively used as a sweetener for food [9]. Molecular structure and formula for sucrose is $C_{12}H_{22}O_{11}$ (Fig. 1).

3. Methods

Isolation of PBMCs

A 2 ml of blood was collected from a normal healthy person in a sodium heparinized vacutainer. The blood was diluted with 1X PBS in 1:1 ratio. Different concentrations of sucrose solutions (10,20,30,4,05,0,60,70,80,90,100%) were prepared. A 4 ml volume of diluted blood was layered on each sucrose solution. The solution was immediately centrifuged at 1000 rpm for 45 min in a cooling centrifuge (4°C). After centrifugation, the tube was removed carefully without disturbing the solution. Four layers could be observed: top reddish clear supernatant, a middle opaque fluid containing the PBMC, clear solution of sucrose and bottom with RBCs. Carefully opaque PBMC layer was transferred to another centrifuge tube and 1X PBS was added up to 8 mL mark. The solution was mixed well and centrifuged at 1000rpm for 10 min in a cooling centrifuge (4°C). The supernatant was discarded and the substance dissolved to 8 mls with 1X PBS.

at 1000 rpm for 10 minutes in a cooling centrifuge (4°C) to remove platelets.

The culture of lymphocytes:

Isolated PBMCs were cultured using 6 mLs of RPMI1640 medium

and incubated overnight at 5% CO2 at 37^0C. After incubation, the tubes were centrifuged at 1000 rpm for 20 minutes and the supernatant was discarded. Pellet was mixed with 500uL of 1X PBS and 50uL of cell suspension was dropped on slides and observed under a microscope.

Cell viability by the hematology analyzer

The isolated cells were analyzed using heme analyzer that checked the number of cells

4. Results

The observed results are shown in the figure 2-4

Figure 2 shows various concentrations of sucrose solutions used for separation of PBMCs of the blood. The 40 % sucrose concentration gave four-layered separation which was the best result.

In figure 3, the four layers shown are: from top to bottom other blood cells (platelets, plasma cells), PBMC, sucrose, and RBC. Isolated PBMCs was observed under heme analyzer for the quality check which showed 75% lymphocytes, 23% monocytes and 2% other cells.

To confirm whether the lymphocytes were separated by this method, isolated PBMCs were cultured using RPMI1640 and incubated overnight at 5% CO2 at 37^0C. Slides were prepared and observed under the microscope to confirm the viable cells (Fig. 4a, 4b).

5. Discussion:

To isolate and purify biomolecules and cell structures, density gradient centrifugation is the commonest technique used. The principle behind this technique is that, in suspension molecules that are denser than the solvent will sediment, while the molecules which are less dense will float by layering liquids of decreased density on a density medium in a centrifuge tube. High-speed ultracentrifuge is used to accelerate this process to separate biomolecules within the density [10, 11, 12].

The commercially available density medium for the separation of layers is ficoll medium which is a polysaccharide. Ficoll gradient centrifugation method for separation of PBMCs is accurate and highly efficient, but it is very expensive. In the present method, commonly available sucrose is used to separate the blood cells. The method is simple and highly economic. Therefore, this method is recommended even in small laboratories with low income for high-throughput sample preparation suitable for various immunological assays.

The resulting PBMC pellet can be used for any immunological and cytotoxicity assays and by this method lymphocytes can be stored for a longer duration of time for any hematological studies or for related scientific research.

6. Conclusion:

Based on the above results we conclude that 40% sucrose is suitable for separation of blood cells or isolation of PBMCs by using low-cost sucrose. Further studies are needed for the development of new gradient media.

Comment [I1]: Explain briefly the importance of developing new gradient media

7. References:

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Comment [I2]: Quote the other authors and avoid “et al” only with one author

Comment [I3]: Use this format of writing references in keeping with other articles published in the Asian Journal

Comment [I4]: Use this format of writing references in keeping with other articles published in the Asian Journal

Comment [I5]: Gerald, O., Y. Masahide, 1963. "Density Gradient Techniques". Chem. Rev. 63 (3): 257–268. doi:10.1021/cr60223a003

Figure 1: Structure of sucrose

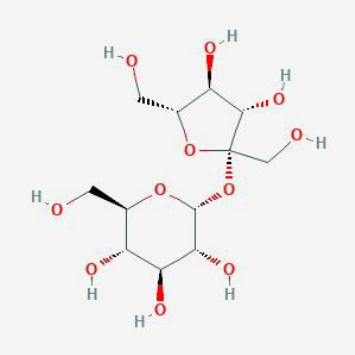
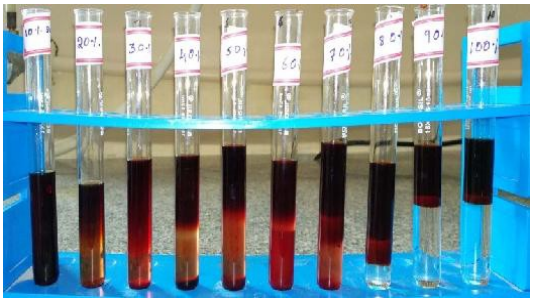


Figure 2: Various concentrations of sucrose solutions from 10% to 100%



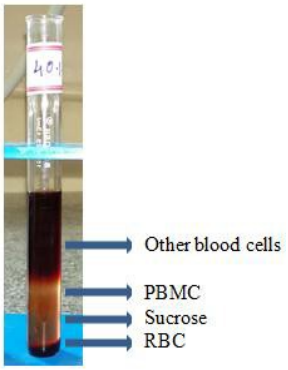


Figure 3: 40% sucrose solution showing 4 different layers of blood cells.

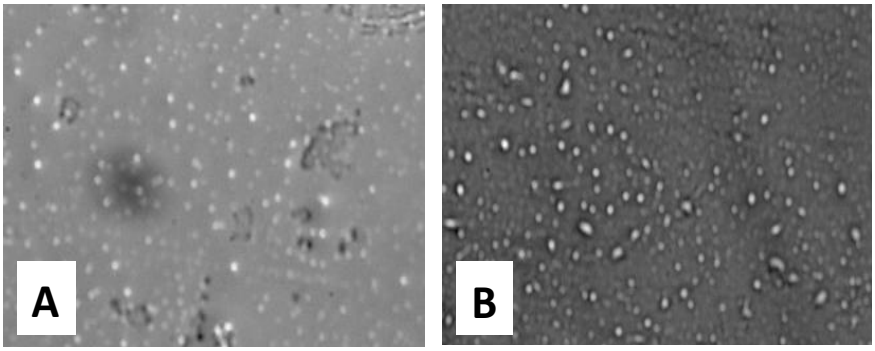


Figure 4: **Image III A&B shows Cultured Cells are dropped on clean slides and viewed under microscope it showed the more viable cells in two different fields.**