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Media and Gradien for Separation of Isolation Cell in Biomedical Research: A Review Article

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ABSTRACT

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Introduction: Isolation of one type or several types of cells from heterogeneous populations is an integral part of modern biological research, routine clinical diagnosis and treatment. The purification of specific cells is essential for basic cell biology research, cellular enumeration in specific pathologies and cell-based regenerative therapies. The main principle of separating any type of cell from a population is to take advantage of one or more specific traits for a particular type of cell.

Discussion: The medium used to separate cells by density gradient centrifugation consists of a gradient that includes the densities of all possible cell types in the mixture. During the centrifugal process, each type of cell will settle to the part of the gradient that has the same density as the cell, namely the isopicnic point. As the above equation, when the density of the medium gradient phase and the cell type are the same, the sedimentation rate is zero, and the cells will settle in that phase instead of the bottom of the tube. Therefore, the cells are only separated by density, regardless of their size. The heavier cells usually become pellets while the lightest cells, along with the dead cells float on the gradient. The gradients used in density-based fractionation are mainly of two types continuous and discontinuous. A discontinuous gradient is made up of different bands with different densities that increase from top to bottom. The separation medium is diluted to a series of different densities and then carefully layered in descending order of density starting from the bottom layer to the top layer. This kind of gradient has a sharp surface between the bands and is therefore used when a sharp band of cells is required on the relevant surface. Continuous gradients are widely used to break up blood cell populations

Conclusion: The separator has three compartments with a closing means which can be operated between the first and second compartments, and between the second and third compartments. Aggregation-inducing density gradient medium (having a density between erythrocyte and MNC densities) was provided in the first (bottommost) compartment, a non-inducing density gradient medium.

Keywords: Isolation Cell, Biomedical, Research, Gradient

INTRODUCTION

Isolation of one type or several types of cells from heterogeneous populations is an integral part of modern biological research, routine clinical diagnosis and treatment.

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The purification of specific cells is essential for basic cell biology research, cellular enumeration in specific pathologies and cell-based regenerative therapies. The main principle of separating any type of cell from a population is to take advantage of one or more specific traits for a particular type of cell[1].

Separation techniques that rely on cell size and density are often used to separate PBMC and bone marrow (BM) cell types. The most commonly used methods in this category include density gradient centrifugation and filtration. They are also called 'mass sorting' methods because

they help isolate relatively large populations of cells in a short period of time. Although the bulk sorting method is an important part of several clinical and biotechnological procedures due to high yields, the purity and homogeneity of the harvested cells is quite low compared to other cell separation procedures[2].

The most commonly used density-based separation method is gradient centrifugation - biological samples are centrifuged in a suitable gradient medium at an appropriate rate until different cell types are fractionated into different layers or phases depending on their respective densities[3].

The medium used to separate cells by density gradient centrifugation consists of a gradient that includes the densities of all possible cell types in the mixture. During the centrifugal process, each type of cell will settle to the part of the gradient that has the same density as the cell, namely the isopicnic point. As the above equation, when the density of the medium gradient phase and the cell type are the same, the sedimentation rate is zero, and the cells will settle in that phase instead of the bottom of the tube. Therefore, the cells are only separated by density, regardless of their size. The heavier cells usually become pellets while the lightest cells, along with the dead cells float on the gradient[2].

DISCUSSION

Density gradient centrifugation is often used as an initial step to enrich a specific cell population before more sophisticated isolation procedures such as FACS, MACS or single cell sorting and extraction of organelles and nucleic acids. The sedimentation rate of cells in suspension is directly proportional to the applied centrifugal force (g) and inversely proportional to the viscosity of the medium: at constant g-force and viscosity, the sedimentation rate will depend on the size of the cell and the difference between the density of the cell and the medium. The relationship between these variables is best explained by the equation below [21]: v = ((d2 * (ρp-ρ0)) / (18 *)) * g; where v = sedimentation rate, d = cell diameter, g = centrifugal force, p = cell density, 0 = medium density, = medium viscosity[4].

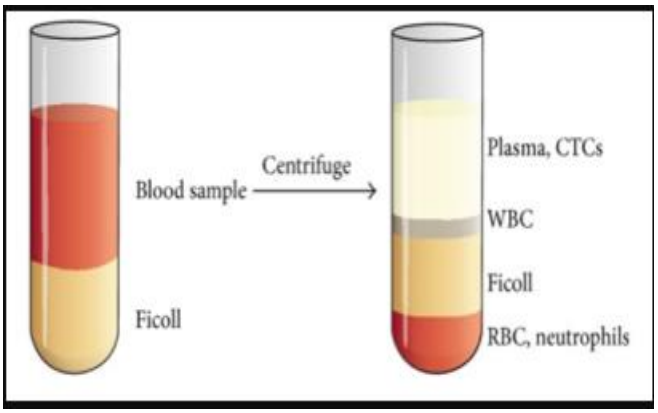

Figure 1. Blood cell fractionation on the ficoll gradient.[4]

The gradients used in density-based fractionation are mainly of two types continuous and discontinuous. A discontinuous gradient is made up of different bands with different densities that increase from top to bottom. The separation medium is diluted to a series of different densities and then carefully layered in descending order of density starting from the bottom layer to the top layer. This kind of gradient has a sharp surface between the bands and is therefore used when a sharp band of cells is required on the relevant surface. Continuous gradients are widely used to break up blood cell populations [1].

A continuous gradient is characterized by a density that increases smoothly from top to bottom - in fact, it can be thought of as being made up of an infinite number of interfaces. Creating a continuous gradient is more complex and requires solutions creating the lowest and highest densities and then blending them to form a linear gradient, spanning the range between the boundaries of the two initial solutions. However, continuous gradients provide greater resolution as the various available densities increase the probability of bands of isopycnic cells at the right density. Furthermore, to separate cells that do not differ much in density, a narrow gradient range can be created. Continuous gradients were used to isolate Leydig cells and intestinal epithelial cells other than BM cells. An ideal gradient medium should have the following properties [5]:

- Adequate density range for isopicnic bands of several cell types
- Physiological ionic strength, pH and osmolarity
- Low viscosity
- Non-toxicity
- Inability to penetrate biological membranes
- Ability to form continuous and discontinuous gradients
- Easily removed from isolated cells
- No effect on downstream testing or culture procedures

Table 1. Some common density gradient media[5].

Medium	Composition	Types of cells and sample reference
Polymorphprep	It contains 13.8% (w/v) sodium diatrizoate and 8.0% (w/v) polysaccharide	Neutrophils (Axis-Shield #1114683 [24])
Polysucrose 400	It is a non-ionic synthetic polymer of sucrose. Combined with sodium diatrizoate to form gradients	WBCs and RBCs Thrombocyte Reticulocytes
Ficoll®	It is a non-ionic synthetic polymer of sucrose. Combined with sodium diatrizoate to form gradients. It has the advantage of being osmotically inert compared to polysucrose	WBCs and RBCs Mononuclear cells and granulocytes Live and dead cells
Percoll®	It is a colloidal suspension of silica particles, 15-30 nm in diameter, coated with polyvinylpyrrolidone (PVP).	All blood cell types (neutrophils, GE Healthcare #17-0891-02 [24]) Bone marrow cell types Liver cells Leydig cells
Optiprep®	It is a 60% (w/v) solution of iodixanol in water	Alveolar cells Spermatozoa Gastric mucosal cells Hepatic stellate cells

Density gradient media with erythrocyte aggregates are Ficoll type media, including e.g. Ficoll™, Ficoll™ PM400, Ficoll-Paque™, Ficoll-Paque™ PLUS (marketed by GE Healthcare, Uppsala, Sweden). Ficoll is a neutral, highly branched, high mass hydrophilic polysaccharide that is readily soluble in aqueous solutions. Another example of aggregation of density gradient media is dextran. In essence, the present invention applies to any prior method and apparatus utilizing a density gradient medium (or media) for the separation of MNCs. Examples of devices include tubular devices, such as tube and syringe type devices[1,6].

The sample medium can be any medium containing the desired MNC in the presence of erythrocytes (in the least amount) and other unwanted cells. Usually, the sample is human peripheral blood, umbilical cord or placental blood, or optional animal blood. Also bone marrow samples may be considered for use in the present invention. As noted above, MNCs consist of a mixture of monocytes and lymphocytes, i.e. leukocytes from which granulocytes are separated and removed. Typically, these represent the MNC content of the hematopoietic stem cells or mesenchymal stem cells of interest (1–2% of MNCs) and can be isolated from the collected and optionally cultured MNC fraction (mesenchymal stem cells)[7].

When applying the method of the present invention to methods and devices using a single density gradient medium, this medium is replaced by two different density gradient media, namely a density gradient medium that does not induce erythrocyte aggregation which is placed over a density gradient medium that induces erythrocyte aggregation. The upper density gradient medium must have a density that is at least no higher than the low density gradient medium. In the event that the density of the two media is the same, or at least essentially the same, the density is chosen such that after centrifugation of the blood sample, the erythrocytes will be separated and positioned under the density gradient medium, whereas the desired MNC will collect at the interface between the original blood sample and the density medium. top gradient[7,8].

The two density gradient media have different densities, that is, the upper density gradient medium has a lower density than the lower density gradient medium. More specifically, the lower density gradient medium should have a density slightly higher than the desired MNC fraction, while the upper density gradient medium should have a slightly less density than the MNC. After centrifugation, the MNC fraction will then be surrounded by two density gradient media, accumulating at the interface between them [9].

The two density gradient media may be coated with each other in direct contact or separately separated by a partitioning means such as a filter, grating or the like which allows passage of the liquid and sample components upon centrifugation. Such a partitioning means may also be provided over or over a layer of the upper density gradient medium to prevent its mixing with the sample applied prior to centrifugation[10,11].

CONCLUSION

The separator has three compartments with a closing means which can be operated between the first and second compartments, and between the second and third compartments. Aggregation-inducing density gradient medium (having a density between erythrocyte and MNC densities) was provided in the first (bottommost) compartment, a non-inducing density gradient medium.

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