

Application Technique in Isolation and Separating Cell for Biomedical Research: A Review Article

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ABSTRACT

INTRODUCTION

The most widely used cell isolation and separation techniques can be broadly classified as based on attachment, morphology (density/size) and antibody binding. High-precision single-cell isolation methods are usually based on one or more of these properties while newer techniques incorporating microfluidics take advantage of several additional cellular characteristics. Recent advances in cell isolation procedures, purity, yield and cell viability have resulted in significant advances in the fields of stem cell biology, oncology and regenerative medicine. The cell isolation procedure can be either positive selection or negative selection the former aims to isolate the target cell type from the entire population, usually with a specific antibody while the latter strategy involves depleting all cell types of the population so as to produce only the remaining target cells. Both types of isolation methods have advantages and disadvantages.

DISCUSSION

The most common application of density gradient centrifugation is blood fractionation. Hence the general protocol for fractionating blood using Ficoll, first collect the blood/buffy coat in an anti-coagulant-coated tube and dilute with an equal volume of PBS. Remove the appropriate volume of Ficoll into a centrifuge tube and carefully layer the diluted blood over the gradient medium. Don't let blood and Ficoll mix. Centrifuge at 400 x g - 500 x g for 30 minutes - 40 minutes at room temperature with the centrifuge brake off. Aspirate the desired cell fraction: Top layer of plasma → platelets; plasma and Ficoll interface → mononuclear cells; layer under Ficoll → granulocytes; pellet → red blood cells. Wash the cells with PBS several times to remove plasma and Ficoll before using the cells for further analysis.

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KEYWORDS

Application; Cell isolation; Technique; Biomedical

INTRODUCTION

The most widely used cell isolation and separation techniques can be broadly classified as based on attachment, morphology (density/size) and antibody binding. High-precision single-cell isolation methods are usually based on one or more of these properties while newer techniques incorporating microfluidics take advantage of several additional cellular characteristics. Recent advances in cell isolation procedures, purity, yield and cell viability have resulted in significant advances in the fields of stem cell biology, oncology and regenerative medicine [1].

The cell isolation procedure can be either positive selection or negative selection the former aims to isolate the target cell type from the entire population, usually with a specific antibody while the latter strategy involves depleting all cell types of the population so as to produce only the remaining target cells. Both types of isolation methods have advantages and disadvantages. Due to the use of specific antibodies targeting specific cell types, positive selection results in higher purity of the desired population. On the other hand, it is more complicated to design antibodies to deplete all non-target cells thereby making negative selection less efficient. Furthermore, cell populations isolated by positive selection can be purified sequentially through several cycles of the procedure, a benefit that negative selective cannot provide. However, positively selected cells carry antibodies and other labeling agents that can interfere with downstream cultures and assays – if

that is a concern, it is preferable to use a negative selection [2].

Isolating a particular cell type from a heterogeneous population depends on the unique properties of that cell type. In this review, we discuss four broad categories of cell isolation techniques based on the following cellular characteristics. Surface charge and adhesion determine the degree of cell attachment to the surface of plastics and other polymers and can be used to separate adherent cells from suspended/free-floating cells. Cell size and density - The physical properties of size and density are usually used for bulk recovery of cells; either by sedimentation, filtration or density gradient centrifugation [3].

DISCUSSION

Different cell types can be distinguished based on their shape, histological staining, selective growth of media, redox potential and other visual and behavioral properties which can then be utilized to isolate the cells. Surface markers, specific binding of surface antigens to antibodies or aptamers can selectively capture specific surface phenotype cells. The captured cells are then detected with the help of scalable probes - usually fluorochromes and magnetic particles with which antibodies/aptamers are labeled [4].

In addition, the above two or more principles can be combined to further enhance isolated cell specificity - typically, the compound technique consists of a label-free method (the first three on the list) together with a label-

coupling method. Selecting a cell isolation method for the experiment depends on the following criteria [3,5]:

Exploitable Cell Characteristics

- The amount of stress - mechanical/chemical/physiological - that the cell type can withstand and still remain viable.

- Purity level and cell yield required.
- Acceptable risk of contamination (zero if cells are required for culture).
- Type of isolation method - is it negative or positive.
- Any downstream application specific requirements, e.g., cell culture, nucleic acid/protein extraction etc.
- Time and cost of labor, reagents, automation (Table 1).

Technique	Principle	Positive/Negative	Purity	Yield
Plastic/Adhesion	Surface Charge and Adhesion	Both	Low	High
Density Gradient Centrifugation	Cell Density	Positive	Low	High
Filtration	Cell Size	Positive	Low	High
FACS	Surface Antigen Binding	Positive	High	Low
MACS	Surface Antigen Binding	Both	High	Medium
Aptamer Binding	Surface Antigen Binding	Positive	High	Low
Selective Growth/Culture	Physiology	Negative	Medium/High	Low/Medium
LCM	Morphology	Positive	High	Low
RBC Rosetting	Size + Surface Antigen	Both	High	Medium
Immuno-LCM	Morphology + Surface Antigen	Positive	High	Low

Table 1: Summarizes the various cell isolation techniques.

General Methodology of Cell Isolation with Density Gradient Medium

The most common application of density gradient centrifugation is blood fractionation. Hence the general protocol for fractionating blood using Ficoll, first collect the blood/buffy coat in an anti-coagulant-coated tube and dilute with an equal volume of PBS. Remove the appropriate volume of Ficoll into a centrifuge tube and carefully layer the diluted blood over the gradient medium. Don't let blood and Ficoll mix. Centrifuge at $400 \times g$ - $500 \times g$ for 30 minutes - 40 minutes at room temperature with the centrifuge brake off. Aspirate the desired cell fraction: Top layer of plasma → platelets; plasma and Ficoll interface → mononuclear cells; layer under Ficoll → granulocytes; pellet → red blood cells. Wash the cells with PBS several times to remove plasma and Ficoll before using the cells for further analysis [5].

Key points to consider ensuring optimal gradient fractionation [4,6]:

- Choice of anticoagulant affects cell quality and final yield
- Preparation of pure monocytes and lymphocytes obtained with EDTA as opposed to heparin which can affect the rate of proliferation
- Citrate is a better choice when cells are needed for DNA and RNA extraction
- Citrate also temporarily increases monocyte yield before de-fibrillation of blood results in low yield
- It is always preferable to fractionate the blood as soon as it is collected. If that is not possible, then the blood should be stored at room temperature for no more than 24 hours. Longer storage time can reduce lymphocyte yield and change the surface immunophenotype.
- Old blood samples should be avoided because they contain a significant amount of red blood cells that lean

on the gradient and make accurate isolation of certain cells difficult.

- Gradient media must be stored at ambient temperature advertisements used before the expiration date as stamped by the manufacturer.

Advantages and limitations of density gradient cell separation [7]

Profit

- The procedure is technically simple and cost-effective; Lab personnel can be easily trained.
- Can be increased or decreased as needed with minimal adjustments.
- The yield of cells obtained, especially for blood samples, is high.

Limitations

- The purity of various obtained cell fractions is low, especially when fractionating blood.
- This procedure is time consuming and has limited results.
- Specific application of density gradient centrifugation
- Fractionation of blood mononuclear cells for clinical and research applications
- Exclusion of dead cells from cell culture harvest - dead cells will come out, and viable cells will be present in culture medium and gradient medium interphase.
- Separation of plasma from blood cells for clinical and diagnostic purposes.
- Special gradient media have been developed to isolate cells from tissues such as liver, pancreas, lungs, testes and intestines.

The compartment size, number of blood samples, and density of the density gradient medium were chosen such that the sample after centrifugation ended up in the middle compartment (which is preferably smaller than the first and third compartments such that the desired purity of the final product can be as high as possible when taken from the

second compartment), i.e., mixed with a minimum of other constituents). Three compartments during centrifugation and can be automatically or manually closed after centrifugation [8].

Gradient density centrifugation (also known as Ficoll, Ficoll-Paque, Biocoll, Pancoll, Hystopaque, and Lymphoprep) is the most common method for separating PBMCs (peripheral blood mononuclear cells) - most specifically lymphocytes and monocytes - from red blood cells (RBCs, erythrocytes). pluriChoose the general method enhanced with a special density gradient medium to also separate all leukocytes with Leuko Spin Media or Platelets with PLT Spin Media. In addition, the pluriMate® centrifuge tube allows for optimal and consistent centrifugation. Ficoll and Ficoll-Paque are Trademarks of GE Healthcare, Biocoll is a Trademark of Biochrom GmbH and Pancoll is a Trademark of PAN-Biotech GmbH [9].

Density gradient centrifugation is usually used for the isolation of certain cell populations from whole blood. This technique takes advantage of the density differences between various leukocytes and the density gradient medium. Consequently, the particular population of cells isolated will depend on the density of the medium used. To isolate mononuclear cells (MNCs) from peripheral blood, cord blood and bone marrow, it is recommended to use media with a density of 1.077 g/mL, such as Lymphoprep™ or Ficoll®. Percoll and ficoll are media that can be used in gradient centrifugation for PBMC isolates. Percoll consists of silica particles coated with PVP (polyvinyl pyrrolidone) which has a density of 1,130 g/mL. Ficoll is a solution containing polysucrose and sodium diatrizoate having a density of 1.077 g/mL. The function of sodium diatrizoate is to optimize the density and osmolarity so that the separation of cells is more efficient. Research that isolated hMSCs (Human mesenchymal stem cells) from bone

marrow stated that the use of ficoll resulted in higher yields than the use of percoll [10].

In this particular application, dilute blood is plated on a density gradient medium, and the sample is centrifuged. Granulocytes and erythrocytes have a higher density than mononuclear cells and during centrifugation they precipitate through a layer of density medium. The less dense mononuclear cells remain at the interface of the plasma density medium. This process is greatly facilitated by performing density gradient centrifugation in the SepMate™ tube. As for the high molecular weight measurements, Ficoll™ PM 70 and Ficoll PM 400 can be used. As mentioned above, the yield of mononuclear cells (MNC) from blood density centrifugation is usually relatively low. For some density gradient media like e.g., Ficoll™ causes its contribution is that the density gradient medium induces clotting, i.e., aggregation of red blood cells (erythrocytes). Although this increases the sedimentation of erythrocytes through the Ficoll layer, it is also believed to cause co-aggregation and co-aggregation of desirable MNCs during the aggregation process, thereby lowering the yield thereof [11].

An example for separating high density gradients can use Ficoll PM 70 and PM 400. Ficoll PM 70 and PM 400 are supplied as dry powders. Ficoll is ideally pH neutral and is the molecule of choice for studying pore size distribution and membrane permeability. Ficoll PM 70 and Ficoll PM 400 have an analogous structure, but differ in molecular weight, and for different applications. The stability of Ficoll is mainly determined by the glycosidic bonds in the sucrose residue. Ficoll does not contain any ionized groups, so the structure does not react under physiological conditions. Stable in alkaline and neutral solutions, but

rapidly hydrolyzes in solution at pH 3, especially at high temperatures. Ficoll can be sterilized by automatic storage at 110°C for 30 minutes in a neutral solution [4,8].

Improved MNC yield by using two different density gradient media, so that the blood sample is first exposed to the first density gradient medium which at least substantially does not induce or increase erythrocyte aggregation. and then come into contact with a second density gradient medium capable of promoting or promoting erythrocyte aggregation. In this way, the co-aggregation of MNCs can be prevented simultaneously as the sedimentation-enhancing effect of erythrocyte aggregation is benefited. Density gradient media which (at least not to a significant degree) do not cause erythrocyte aggregation, as well as media that induce aggregation are known to those skilled in the art. Density gradient media not aggregating erythrocytes are Percoll type media, including e.g., Percoll™ and Percoll™ PLUS (marketed by GE Healthcare, Uppsala, Sweden). Percoll consists of colloidal silica particles 15 nm - 30 nm in diameter which have been coated with polyvinylpyrrolidone. Other examples of non-aggregated density gradient media include sucrose, and iodinated density gradient media, such as iodixanol, nycodenz and metrizamide [2,8].

CONCLUSION

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REFERENCES

1. Aiello AE (2010) Cytomegalovirus antibodies as a marker of immunosenescence in the Detroit neighborhood health study. 9th International Conference on Urban Health. New York Academy of Medicine.

2. Uddin M (2011) Post-traumatic stress disorder is associated with immunosenescent T cell phenotypes in the Detroit neighborhood health study. 3rd North American Congress of Epidemiology. Montreal, QC.
3. Mallone R, Mannering SI, Brooks-Worrell BM, et al. (2011) Isolation and preservation of peripheral blood mononuclear cells for analysis of islet antigen-reactive T cell responses: Position statement of the T-cell workshop committee of the immunology of diabetes society. *Clinical & Experimental Immunology* 163(1): 33-49.
4. Duvigneau JC, Hartl RT, Teinfalt M, et al. (2003) Delay in processing porcine whole blood affects cytokine expression. *Journal of Immunological Methods* 272(1-2): 11-21.
5. Uddin M, Aiello AE, Wildman DE, et al. (2010) Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proceedings of the National Academy of Sciences* 107(20): 9470-9475.
6. Colter DC, Class R, DiGirolamo CM, et al. (2000) Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow. *Proceedings of the National Academy of Sciences* 97(7): 3213-3218.
7. Segman RH, Shefi N, Goltser-Dubner T, et al. (2005) Peripheral blood mononuclear cell gene expression profiles identify emergent post-traumatic stress disorder among trauma survivors. *Molecular Psychiatry* 10(5): 500-513.
8. Debey S, Schoenbeck U, Hellmich M, et al. (2004) Comparison of different isolation techniques prior gene expression profiling of blood derived cells: Impact on physiological responses, on overall expression and the role of different cell types. *The Pharmacogenomics Journal* 4(3): 193-207.
9. Šonka J, Stöhr M, Vogt-Schaden M, et al. (1983) Isopycnic density-gradient centrifugation: A separation parameter which improves flow cytometric measurements on heterogeneous tumors. *Cytometry: The Journal of the International Society for Analytical Cytology* 4(2): 141-149.
10. Ulmer AJ, Flad HD (1979) Discontinuous density gradient separation of human mononuclear leucocytes using percoll[®] as gradient medium. *Journal of Immunological Methods* 30(1): 1-10.
11. Weiswald LB, Bellet D, Dangles-Marie V (2015) Spherical cancer models in tumor biology. *Neoplasia* 17(1): 1-15.