FACULTY OF MEDICAL SCIENCES

LT.9. Cell separation, dispersion and disruption of tissue

Outline

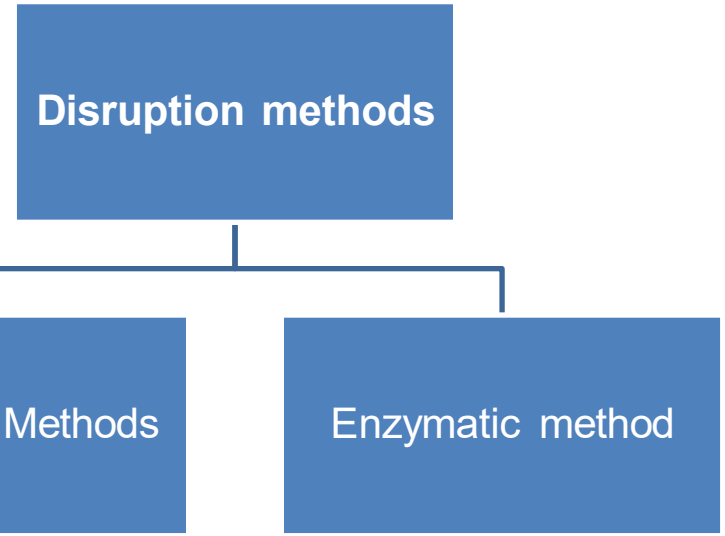
1. Disaggregation /Dispersion of tissues
    a. Mechanical methods
    b. Enzymatic methods
2. Cell separation
3. Cell separation techniques
4. Separation methods

Disaggregation of tissues

Initiation of cell culture requires cell disruption and disaggregation for converting it into single cells and to remove cellular interconnection.

Methods of cell disaggregation

1. Mechanical Methods
2. Enzymatic methods



Mechanical methods:

- Sieve of varying Pore size and mortar pestle can be used.
- These methods are harsh and has low yield.
- Pressing the tissue pieces through a series of sieves with a gradual reduction in the mesh size.
- Forcing the tissue fragments through a syringe and needle.

Enzymatic methods:

•Various enzymes such as collagenase, trypsin, pronases are commonly used for tissue

•Disaggregation.

•Depending upon types of tissues to be disaggregated and enzymes used, disruption protocol differs.

•Enzymatic methods are mild and has high yield of viable cells after disruption. However, enzymes are expensive.

Follow this link for step wise protocol:

https://biocyclopedia.com/index/biotechnology/animal_biotechnology/animal_cell_tissue_and_organ_culture/biotech_isolation_animal_material.php

Cell separation

What is cell separation?

Cell separation, also commonly referred to as cell isolation or cell sorting, is a process to isolate one or more specific cell populations from a heterogeneous mixture of cells.

Principles behind separation of cell types by various method

Cells can be separated on the basis of any one or in combination of following separation principle:

•Cell size

•Cell density

• Electrostatic and hydrophobic qualities

• The expression of cell-specific surface markers

•Adherence to tissue culture plastic

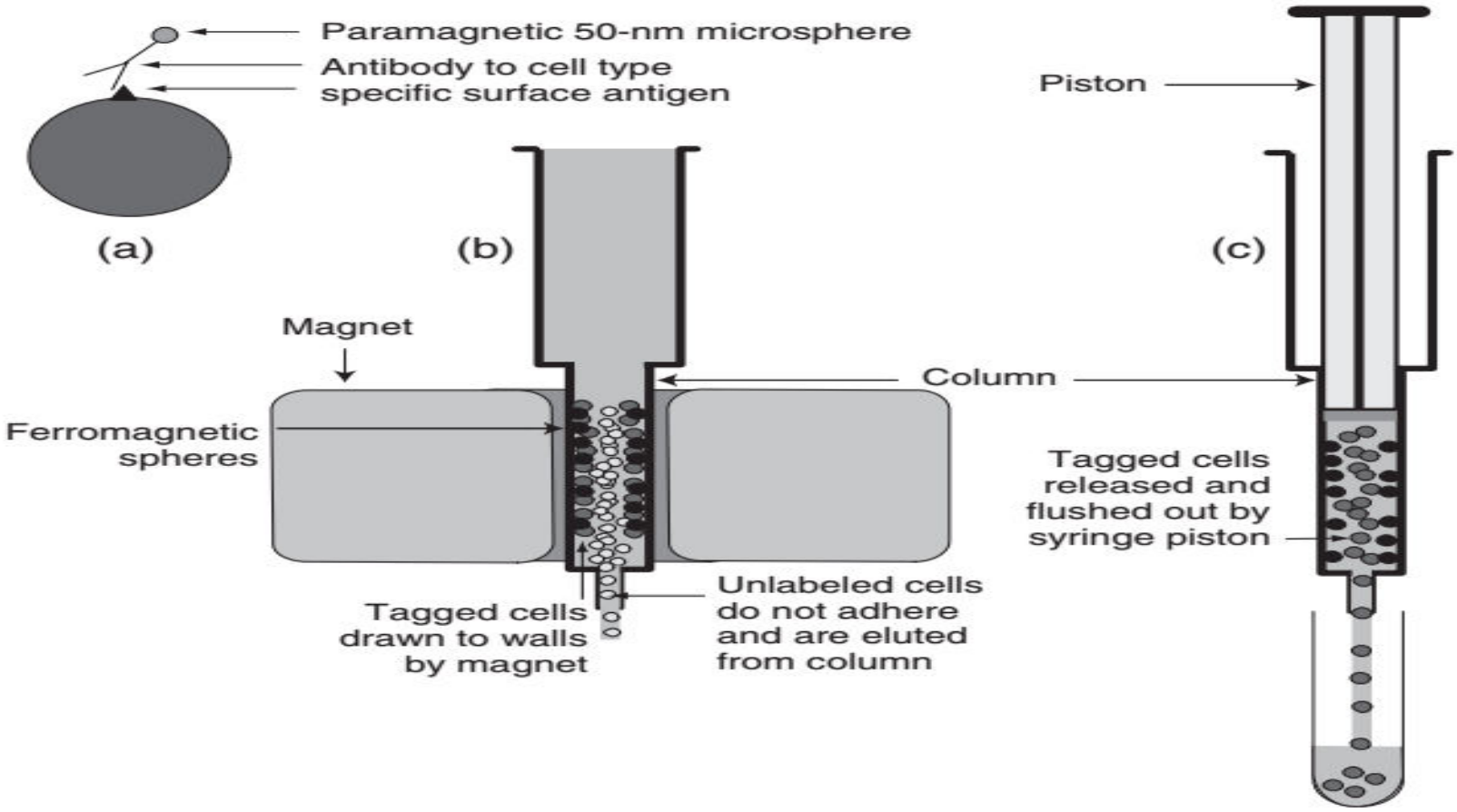
•Immunological characteristics of cells

Separation methods and techniques for separation of cells

a. Immunomagnetic cell separation
b. Fluorescence-activated cell sorting (FACS)
c. Density gradient centrifugation
d. Immunodensity cell isolation
e. Microfluidic cell sorting
f. Adhesion and filtration
g. Centrifugal elutriation
h. Micromanipulation

Separation methods: Immunomagnetic cell separation

Magnetic cell separation, also known as immunomagnetic cell separation uses a specific antibody, raised against a cell surface epitope, conjugated to ferritin beads (Dynabeads, from Invitrogen—Dynal) or microbeads.



Adapted from Animal cell culture, Freshney

Separation methods: Density gradient centrifugation

•This technique is used to separate biological particles of similar size but differing density, ultracentrifugation with pre-formed or self establishing density gradient is used.

•The density centrifugation is continued until the buoyant density of the particle of interest and density of the gradient are equal.

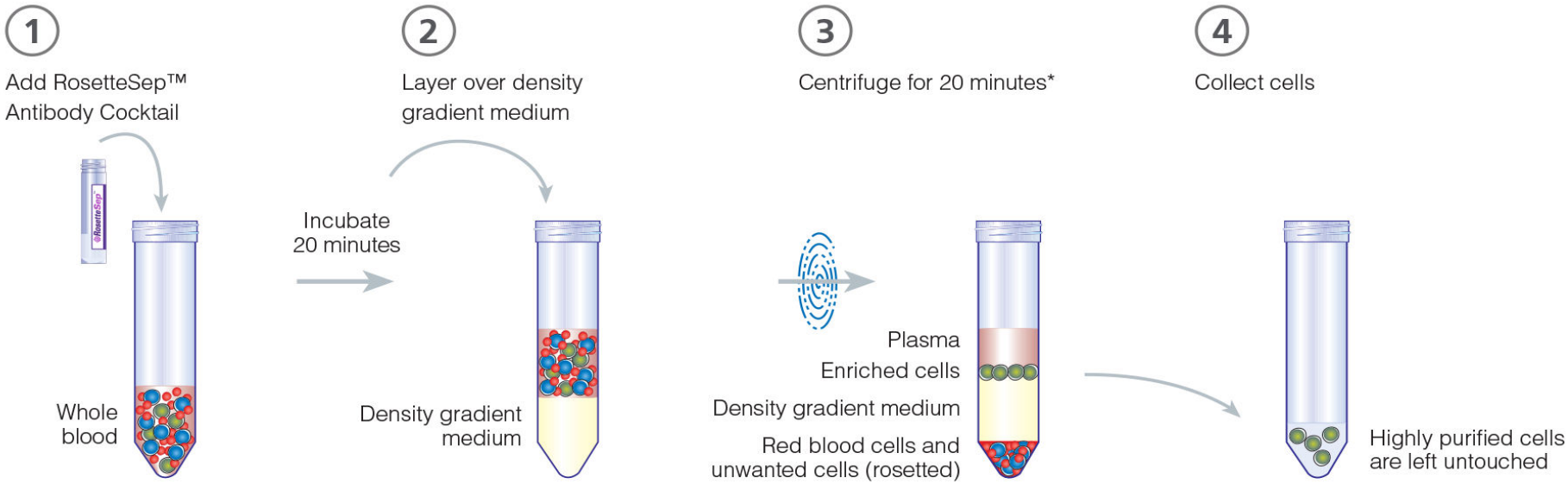
•The cells sediment in a density gradient to an equilibrium position equivalent to their own density.

•Serum albumin, dextran, Ficoll, metrizamide, and Percoll are commonly used to prepare gradient

•The sample is layered on top of a density gradient medium before being centrifuged. During centrifugation, each cell type will sediment to its isopycnic point, which is the place in the medium gradient where the density of the cells and medium are equal.

Separation methods: Immunodensity cell separation

Immunodensity cell separation, also referred to as erythrocyte rosetting, is a negative selection method that uses a combination of antibody-based labeling and density gradient centrifugation



*Use SepMate™ to reduce centrifugation time to 10 minutes with brake on.

Adapted from https://www.stemcell.com/products/brands/rosettesep-immunodensity-cell-separation.html

Separation methods: Centrifugal elutriation

•Centrifugal elutriation is a velocity sedimentation method that separates cells on the basis of size, shape, and density.

•Charles Lindberg (1930) first used this technique to separate cell subpopulations from a variety of biological systems.

•Cells are separated according to their rate of sedimentation in a gravitational field where the liquid containing the cells is made to flow against the gravitational force.

•Cells are thus subjected to two opposing forces within the separation chamber, a centrifugal force generated by the spinning rotor and the counterflow of the fluid in the opposite direction.

•. Each cell migrates to a zone in the chamber where its sedimentation velocity is exactly balanced by the flow rate of the fluid in the opposite direction.

•Cells of different size tend to accumulate in discrete sections of the chamber

•Cells of a particular size that have accumulated in a particular area of the chamber can be eluted (elutriated) by either increasing the flow rate of the liquid or decreasing the centrifugal force (rotor speed).

This principle is described in ***Laboratory Techniques in Biochemistry and Molecular Biology***
Volume 18, 1988, Pages 91-106

Separation methods: Fluorescence activated cell sorting (FACS)

•Fluorescence-activated cell sorting (FACS) is a specialized type of flow cytometry.

•A *fluorescence-activated cell sorter* is an instrument that uses the emission signals from each cell to sort the cell into one of four sample collection tubes and a waste reservoir.

•It provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell.

•. Propidium iodide or chromomycin A3 for DNA or a fluorescent antibody are used as fluorescent molecules.

•The fluorescence emission excited by the laser is detected by a second photomultiplier tube.

Schematic working and principle of FACS

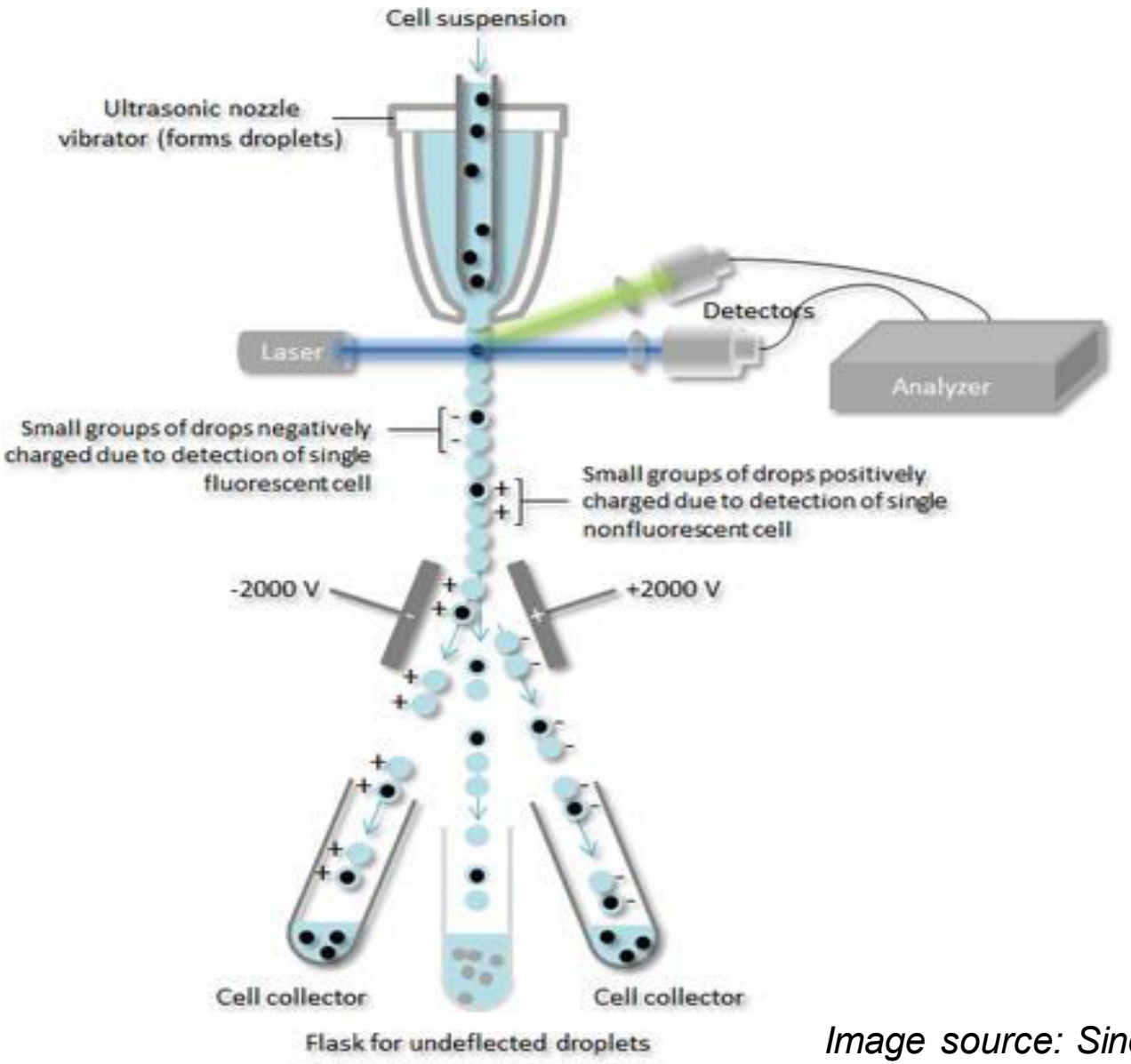


Image source: Sino Biological

Advantages and disadvantages of FACS

•Immunomagnetic cell separation is a much faster and simpler procedure than FACS, and is often the preferred cell isolation method for common cell types. FACS has several advantages over immunomagnetic cell separation including the ability to:

•Sort single cells

•Isolate cells based on intracellular markers (e.g. GFP)

•Isolate cells based on surface marker expression levels

•Sort complex cell types with multiple markers at higher purity

MCQs

1.Erythrocyte rosetting is a

a. a combination of antibody-based labeling and density gradient centrifugation also known as immunodensity cell separation
b. Immunodensity cell separation doesn't require any specialized equipment beyond a centrifuge
c. mmunodensity cell separation doesn't require any specialized equipment beyond a centrifuge All above applies
d. All of the above

2.Fluorescence-activated cell sorting (FACS)

a. It is a specialized type of cell sorting
b. It is based upon the specific light scattering and fluorescent characteristics of each cell
c. Propidium iodide or chromomycin A3 are used as fluorescent dye
d. All of the above

3.The separating principle of filtration is based on

a. Size and shape
b. Differences in adherence phenomena
c. Both (a) and (b)
d. Neither (a) nor (b)

References & further reading

1. https://labs.biotecnika.org/blogs/research-tips/facs-guide-researcher-complete-guide
2. https://www.sciencedirect.com/science/article/pii/S0075753508706302
3. https://www.beckman.com/resources/fundamentals/principles-of-centrifugation/centrifugal-elutriation
4. https://www.beckman.com/support/faq/scientific/what-is-centrifugal-elutriation