Cell fractionation

Cell fractionation is a method for **obtaining parts of a cell capable of independent function**. It is used to separate organelles and macromolecules for subsequent analysis of their composition and functions. Individual macromolecules can then be further isolated and analyzed, e.g. to determine protein disorder. The fractionation procedure consists of two parts: **cell homogenization** and **component purification**.

Cell homogenization

Homogenization of cells is a process in which the **cell membrane is disrupted** and **cell components are released** into the solution. We can achieve it by several methods. Roughly, they are divided between **rough** and **subtle methods**. Gentle methods are used, for example, for cell cultures. In contrast, coarse methods are used for stronger structures that require some degree of mechanical damage, such as connective or skin tissue.

Rough methods

Cell homogenizer Emulsiflex

Ball homogenization
: In this method, we use small metal or glass balls, which are mixed with the cells in a certain ratio. Subsequent mixing or shaking causes the balls to crush the membranes. The method is very gentle, but unfortunately time-consuming.

Ultrasound
: Membranes are crushed by repeated ultrasonic shocks. The disadvantage of this method is that it generates a high amount of heat, which can damage the function of the cell components and thus invalidate the entire procedure. Therefore, a frozen preparation is used for protein isolation.

High pressure
: In this method, the cells are crushed by high pressure. An example of this would be pushing cells through a narrow opening where the cells burst as they pass through. For this we can use, for example, the French press (https://en.wikipedia.org/wiki/French_pressure_cell_press).

Gentle blending
: Frozen tissue culture can be mixed with a gentle blender to mechanically break the cell wall and membrane. Alternatively, we can grind the frozen sample mechanically.

Subtle methods

Repeated freezing and thawing
: The method is particularly effective for animal cells. Cells are repeatedly frozen (e.g. with liquid nitrogen) and allowed to thaw. Since ice has a larger volume than water, ice crystals break through cell membranes when frozen and release their contents.

Enzymatic digestion
: With the use of enzymes specific for each cell culture, the components of the cell membrane or wall will be digested. It is often used in combination with other methods. Mostly used for bacteria, yeast, *fungi*, *algae*, even animal cells.

Detergent
: With the help of a detergent, the cell membrane is dissolved. Again, it is often used in conjunction with other methods to achieve the desired effect. Suitable for animal cells.

Osmotic lysis
: In a hypotonic solution, the cell absorbs water, swells and eventually bursts, releasing its contents into the environment. Again, a suitable method for animal cells that do not have a cell wall.

Component purification

We can separate non-homogenized cells from the rest by coarse filtration (e.g. through gauze) Centrifugation and ultracentrifugation are mainly used for the flow-through separation of components . Based on the **different size and volume of** individual organelles and macromolecules, sedimentation in centrifuges takes place at **different speeds**. Thanks to the extreme gravitational overload, individual macromolecules also sediment.

First, large components such as cell nuclei sediment. At higher speeds mitochondria, then fragments of endoplasmic reticulum. At higher speeds and longer centrifugation time, small vesicles settle, followed by ribosomes and smaller particles. All these fractions **are not pure**, at first , but they can be purified by separating the sediment **resuspending** it and **centrifuging it again**. However, it is usually necessary to repeat the whole process several times.

Links

Related articles

- Centrifugation

External articles

- French press (https://en.wikipedia.org/wiki/French_pressure_cell_press)

Used literature

- NINFA, Alexander – BALLOU, David – BENORE, Marilee. *Fundamental Laboratory Approaches for Biochemistry and Biotechnology.* 1.. edition. John Wiley & Sons, 2009. 480 pp. ISBN 9780470087664.
- ALBERTS, Bruce. *Molecular Biology of the Cell.* 1.. edition. Garland, 2004. ISBN 9780815332183.
- Geno Technology, Inc. *Cell Disruption Techniques* [online]. [cit. 2020-09-28]. <https://info.gbiosciences.com/blog/cell-disruption-techniques-sonication-dounce-homogenizer-more>.