Techniques used for cell lysis / cell disruption

Mechanical Methods

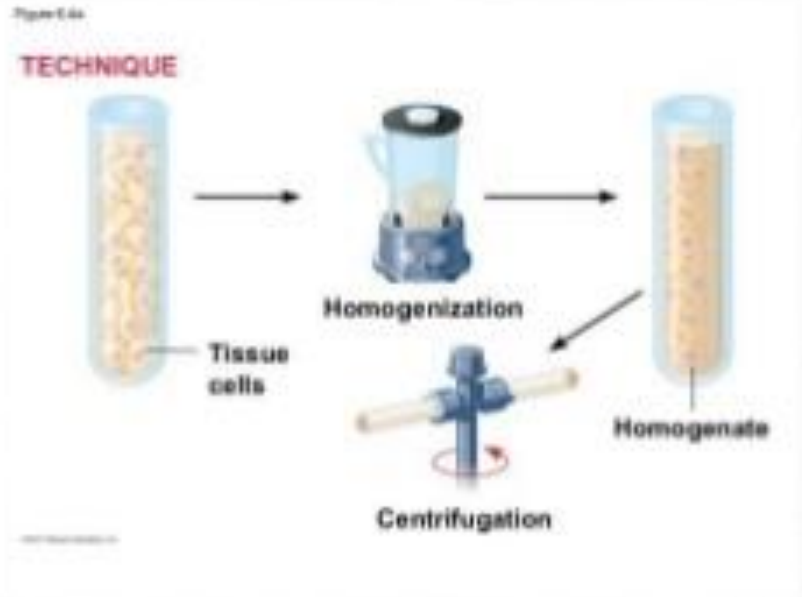
- Mechanical methods are those methods which required some sort of **force to separate out intracellular protein** without adding chemical or enzyme
  1. Mortar & pastel/grinding
  2. Blender
  3. Bead beating
  4. Ultra sonication
  5. Homogenization

Mortar & Pestle

- Just give the cells a good old grinding.
- This does not have to be in suspension and is often done with plant samples frozen in liquid nitrogen.
- When the material has been disrupted, metabolites can be extracted by adding solvents.

Blenders

- The use of blenders (high speed can be used to disrupt cell walls.
- This is the same process used by centrifugation, which separates or concentrates materials suspended in a liquid medium



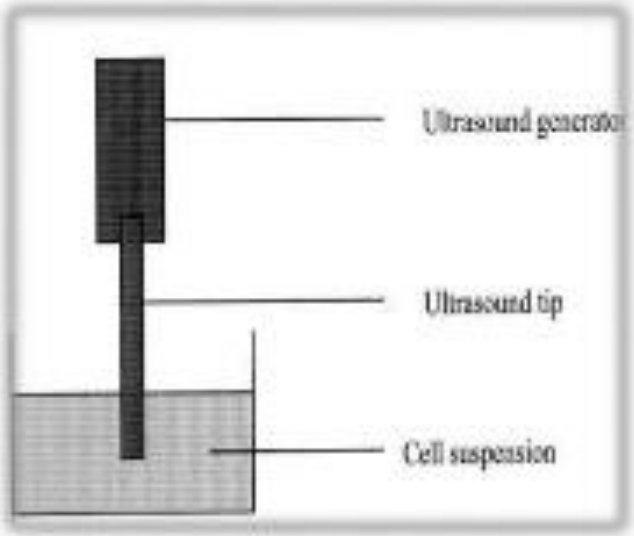
Bead beating

- Glass or ceramic beads are used to crack open cells
- but this kind of mechanical shear is gentle enough to keep organelles intact.
- It can be used with all kinds of cells, just add beads to an equal amount of cell suspension and vortex

Ultra sonication

- Ultrasonic homogenizers work by inducing vibration in a titanium probe that is immersed in the cell solution.
- A process called cavitation occurs, in which tiny bubbles are formed and explode, producing a local shockwave and disrupting cell walls by pressure change.
- This method is very popular for plant and fungal cells but comes at a disadvantage: It's very loud and has to be performed in an extra room

Ultra sonication



Homogenization

- Liquid-based homogenization is the most widely used cell disruption technique for small volumes and cultured cells.
- Cells are lysed by forcing the cell or tissue suspension through a narrow space
- Homogenizers use shearing forces on the cell similar to the bead method. Homogenization can be performed by squeezing cells through a tube that is slightly smaller than beads beating.

Non Mechanical Methods

- Non mechanical methods are further divided into three class which are following
- Physical methods
  1. Freeze thaw
  2. Microwave / Thermolysis
  3. Osmatic shock
  4. Electric discharges
- Chemical methods
- Enzymatic methods

Freeze Thaw

- This method used when working with soft plant material and algae.
- freezing is used to achieve cell disruption via a series of freezing and thawing cycles.
- Freezing forms ice crystals, which expand upon thawing, and this ultimately causes the cell wall to rupture.

Microwave

- Microwave (along with autoclave and other high temperature methods) are used to disrupt the bonds within cell walls, and also to denature proteins. This is a somewhat risky method, as the excess heat can quickly damage the rest of the cell.

Osmatic shock

- Through the process of osmosis, water can be moved into the cell causing its volume to increase to the point that it bursts.
- Note that this method can only work with animal cells and protozoa, since they do not have cell walls.

Electrical Discharges

- It is also possible to achieve cell disruption via electrical discharges in mammalian cells.
- Cells that are bounded by plasma membranes and, unlike plant cells, have no cell wall.
- This method allows researchers to examine secretion by exocytosis, which is a process during which the membrane-bounded sphere (intracellular vesicle) shifts to and fuses with the plasma membrane

Chemical Method

- Often used with plant cells (and sometimes in combination with shearing), organic solvents such as toluene, ether, benzene, methanol, surfactants, and phenyl ethyl alcohol DMSO can be used to permeate cell walls.
- EDTA can be used specifically to disrupt the cell walls of gram negative bacteria, whose cell walls contain lipopolysaccharides that are stabilized by cations like Mg^{2+} and Ca^{2+}. EDTA will chelate the cations leaving holes in the cell walls.

Enzymatic methods

- Enzymes such as beta(1-6) and beta(1-3) glycanases, proteases and mannase can be used to disrupt the cell wall.
- This method is particularly useful for isolating the cell without the wall (protoplast).