CULTURING OF CELLS
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BIOTECHNOLOGY

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CELL CULTURE

- Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favourable artificial environment.

PRIMARY CELL CULTURE

- Primary culture refers to the stage of the culture after the cells are isolated from the tissue and proliferates under the appropriate conditions until they occupy all the available substrate.
- At this stage the cells have to be sub cultured by transferring them to a new culture vessel with fresh growth medium for continuous growth.

COMMON PRIMARY CELL TYPES USED

- Epithelial cells
- Endothelial cells
- Fibroblasts
- Osteoblasts
- Hepatocytes
- Blood cells
- Chondrocytes
- Adipocytes
- Keratinocytes
- Astrocytes

APPLICATIONS OF PRIMARY CELL CULTURE

- This is increasingly being used as a major tool in cellular and molecular biology , providing excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging, signaling studies), the effects of drugs and toxic compounds on the cells and mutagenesis and carcinogenesis
- Used in drug screening as well as for the development of biological compounds (such as: vaccines, therapeutic proteins) on a large scale.
- Used in tissue or organ replacement, extensively used in 3D bio printing , stem cell therapy , etc.

PRIMARY CELL CULTURE: 3 TECHNIQUES

- Mechanical disaggregation.
- Primary explant technique.
- Enzymatic disaggregation.

DURING EXPERIMENT FOLLOWING CRITERIA ARE CONSIDERED FOR EFFICIENT DEVELOPMENT FOR PRIMARY CULTURE

- Embryonic tissues rather than adult tissues are preferred for primary cultures. This is due to the fact that the embryonic cells can be disaggregated easily and yield more viable cells, besides rapidly proliferating in vitro.
- The quantity of cells used in the primary culture should be higher since their survival rate is substantially lower (when compared to subcultures).
- The tissues should be processed with minimum damage to cells for use in primary culture. Further, the dead cells should be removed.
- Selection of an appropriate medium (preferably a nutrient rich one) is advisable. For the addition of serum, fetal bovine source is preferred rather than calf or horse serum.
- It is necessary to remove the enzymes used for disaggregation of cells by centrifugation.

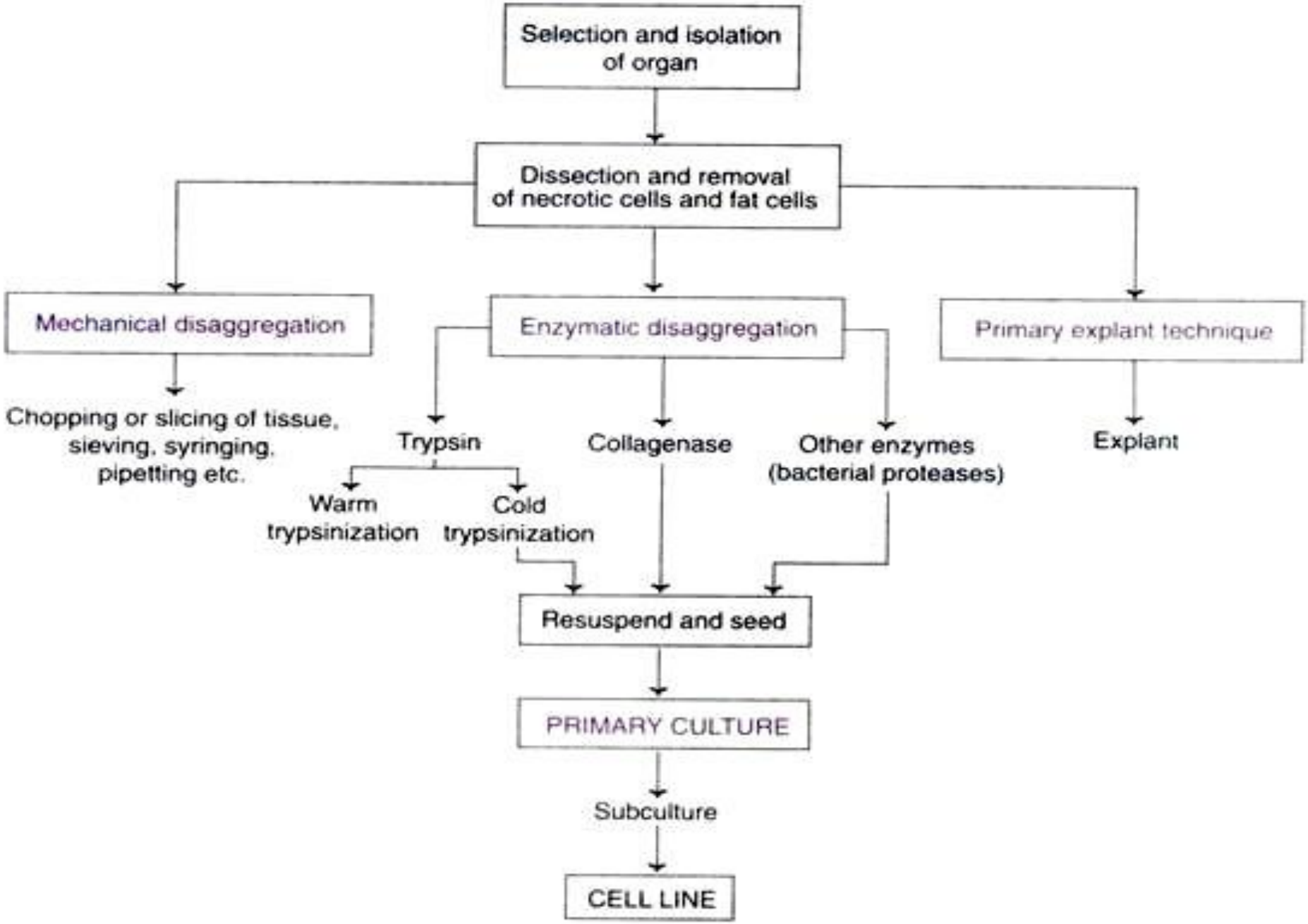


Fig. 36.1 : Different techniques used for primary culture.

TECHNIQUE # 1. MECHANICAL DISAGGREGATION:

- This technique basically involves careful chopping or slicing of tissue into pieces and collection of spill out cells.
- **The cells can be collected by two ways:**

i. Pressing the tissue pieces through a series of sieves with a gradual reduction in the mesh size.

ii. Forcing the tissue fragments through a syringe and needle.

- Although mechanical disaggregation involves the risk of cell damage, the procedure is less expensive, quick and simple. This technique is particularly useful when the availability of the tissue is in plenty, and the efficiency of the yield is not very crucial. It must however, be noted that the viability of cells obtained from mechanical techniques is much lower than the enzymatic technique.

TECHNIQUE # 2. PRIMARY EXPLANT TECHNIQUE:

- The primary explant technique was, in fact the original method, developed by Harrison in 1907. This technique has undergone several modifications, and is still in use. The simplified procedure adopted for primary explant culture is depicted in Fig. 36.4, and briefly described below

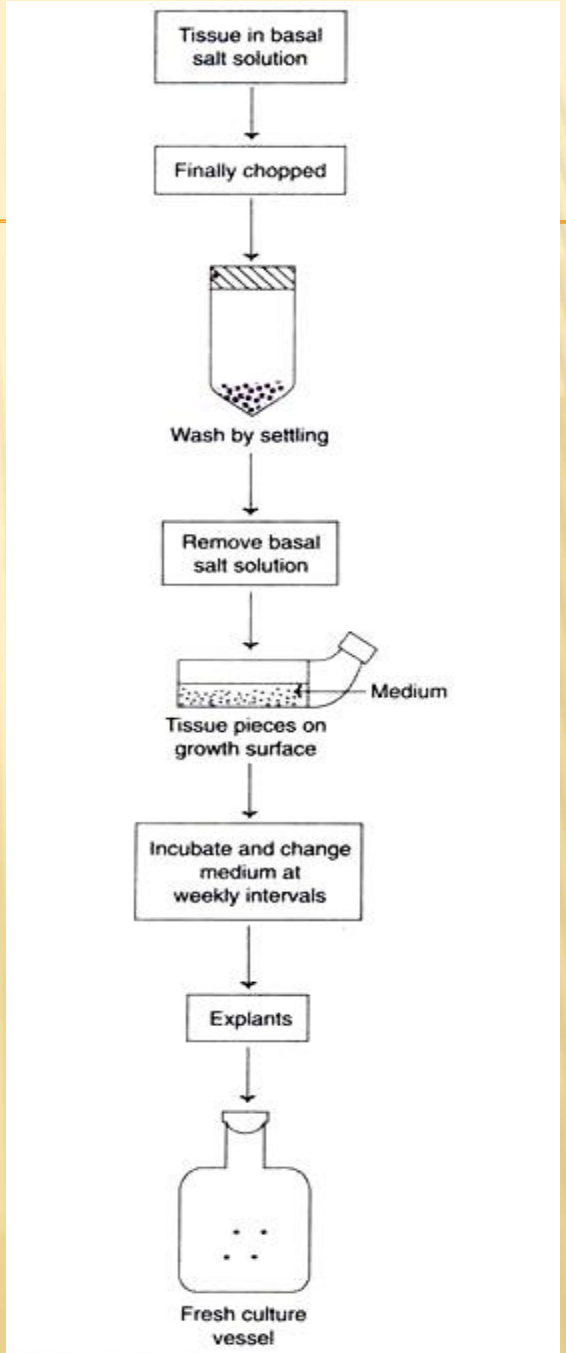


Fig. 36.4 : Primary explant technique for primary culture.

- The tissue in basal salt solution is finely chopped, and washed by settlings. The basal salt solution is then removed. The tissue pieces are spread evenly over the growth surface. After addition of appropriate medium, incubation is carried out for 3-5 days. Then the medium is changed at weekly intervals until a substantial outgrowth of cells is observed. Now, the explants are removed and transferred to a fresh culture vessel.
- The primary explant technique is particularly useful for disaggregation of small quantities of tissues (e.g. skin biopsies). The other two techniques mechanical or enzymatic disaggregation however, are not suitable for small amounts of tissues, as there is a risk of losing the cells.
- The limitation of explant technique is the poor adhesiveness of certain tissues to the growth surface, and the selection of cells in the outgrowth. It is however, observed that the primary explant technique can be used for a majority of embryonic cells e.g. fibroblasts, myoblasts, epithelial cells, glial cells.

TECHNIQUE # 3. ENZYMATIC DISAGGREGATION:

- Enzymatic disaggregation is mostly used when high recovery of cells is required from a tissue.
- Disaggregation of embryonic tissues is more efficient with higher yield of cells by use of enzymes. This is due to the presence of less fibrous connective tissue and extracellular matrix.
- Enzymatic disaggregation can be carried out by using trypsin, collagenase or some other enzymes.

Disaggregation by trypsin:

- Many workers prefer to use crude trypsin rather than pure trypsin for the following reasons:

i. The crude trypsin is more effective due to the presence of other proteases

ii. Cells can tolerate crude trypsin better.

iii. The residual activity of crude trypsin can be easily neutralized by the serum of the culture media (when serum-free media are used, a trypsin inhibitor can be used for neutralization)..

- Disaggregation of cells can also be carried out by using pure trypsin which is less toxic and more specific in its action. The desired tissue is chopped to 2-3 mm pieces and then subjected to disaggregation by trypsin. There are two techniques of trypsinization-warm trypsinization and cold trypsinization

Warm trypsinization :

- This method is widely used for disaggregation of cells. The chopped tissue is washed with dissection basal salt solution (DBSS), and then transferred to a flask containing warm trypsin (37° C). The contents are stirred, and at an interval of every thirty minutes, the supernatant containing the dissociated cells can be collected. After removal of trypsin, the cells are dispersed in a suitable medium and preserved (by keeping the vial on ice).
- The process of addition of fresh trypsin (to the tissue pieces), incubation and collection of dissociated cells (at 30 minutes intervals) is carried out for about 4 hours. The disaggregated cells are pooled, counted, appropriately diluted and then incubated.

Cold trypsinization:

- This technique is more appropriately referred to as trypsinization with cold pre-exposure. The risk of damage to the cells by prolonged exposure to trypsin at 37°C (in warm trypsinization) can be minimized in this technique.
- After chopping and washing, the tissue pieces are kept in a vial (on ice) and soaked with cold trypsin for about 6-24 hours. The trypsin is removed and discarded. However, the tissue pieces contain residual trypsin. These tissue pieces in a medium are incubated at 37°C for 20-30 minutes. The cells get dispersed by repeated pi-pettings. The dissociated cells can be counted, appropriately diluted and then used.

Limitations of trypsin disaggregation:

- Disaggregation by trypsin may damage some cells (e.g. epithelial cells) or it may be almost ineffective for certain tissues (e.g. fibrous connective tissue). Hence other enzymes are also in use for dissociation of cells

Disaggregation by collagenase:

- The enzyme collagenase (usually a crude one contaminated with non-specific proteases) can be effectively used for the disaggregation of several tissues (normal or malignant) that may be sensitive to trypsin.
- Highly purified grades of collagenase have been tried, but they are less effective when compared to crude collagenase. The important stages in collagenase disaggregation, depicted in , are briefly described hereunder.
- The desired tissue suspended in basal salt solution, containing antibiotics is chopped into pieces. These pieces are washed by settling, and then suspended in a complete medium containing collagenase. After incubating for 1-5 days, the tissue pieces are dispersed by pipetting. The clusters of cells are separated by settling. The epithelial cells and fibroblastic cells can be separated.
- Collagenase disaggregation has been successfully used for human brain, lung and several other epithelial tissues, besides various human tumors, and other animal tissues. Addition of another enzyme hyaluronidase (acts on carbohydrate residues on cell surfaces) promotes disaggregation

- Collagenase in combination with hyaluronidase is found to be very effective for dissociating rat or rabbit liver. This can be done by per-fusing the whole organ in situ. Some workers use collagenase in conjunction with trypsin, a formulation developed in chick serum, for disaggregation of certain tissues.

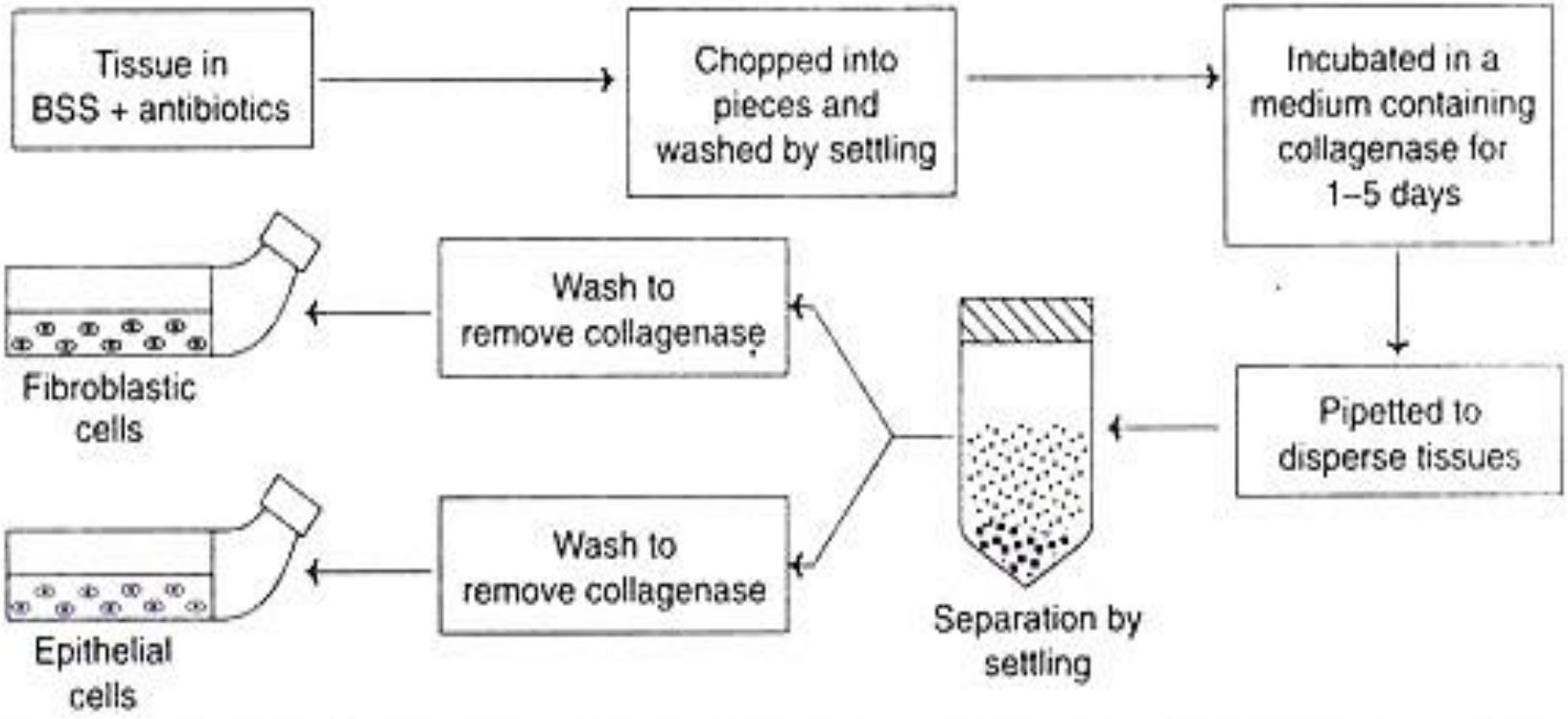


Fig. 36.3 : Important stages in collagenase disaggregation of tissue for primary culture (BSS–Basal salt solution).

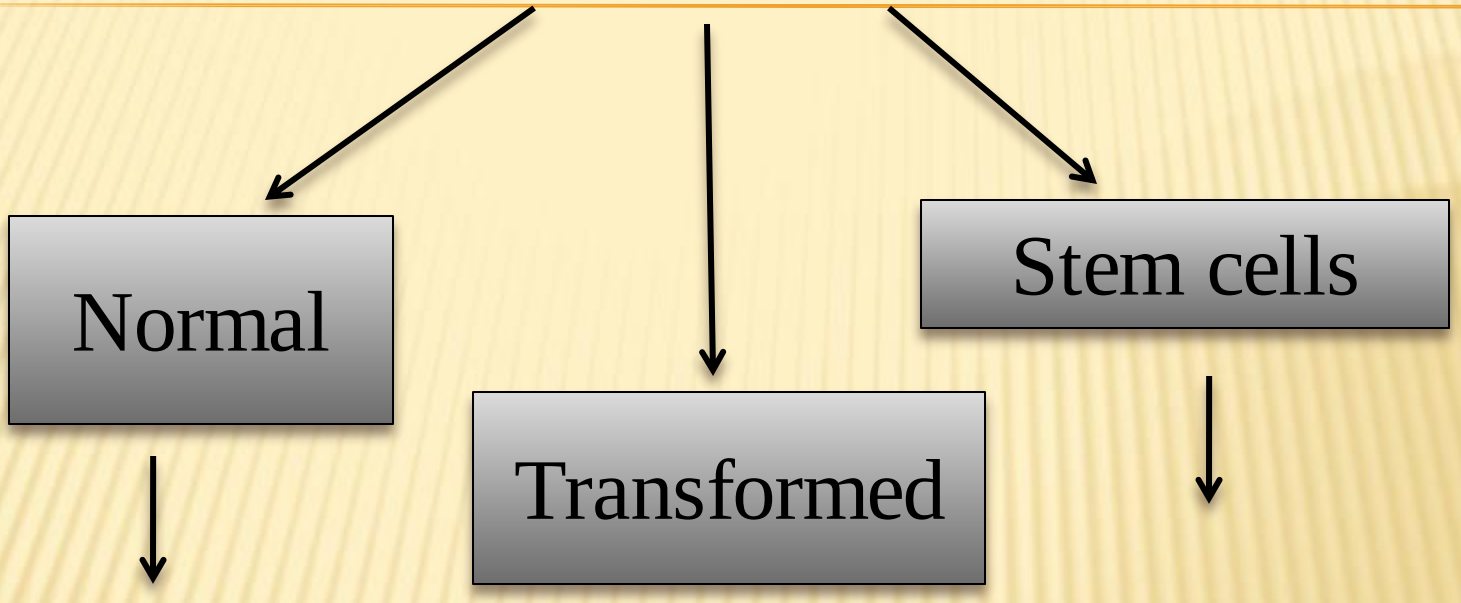
Use of other enzymes in disaggregation:

- Trypsin and collagenase are the most widely used enzymes for disaggregation. Certain bacterial proteases have been used with limited success. Besides hyaluronidase, neuraminidase is also used in conjunction with collagenase for effective degradation of cell surface carbohydrates.

CELL LINE

- A cell line is a permanently established cell culture that will proliferate indefinitely given appropriate fresh medium and space.
- A cell culture developed from a single cell and therefore consisting of cells with a uniform genetic make-up.
- There is presence of several cell linkage either similar or distinct.
- Generally stem cells are used in this culture.
- After the sub culture, the primary culture becomes cell line & may be propagated & sub cultured several times.
- Some species particularly rodents give rise to lines relatively easily
- No cell line have been produced from avian tissues and the establishment of cell lines from human tissue if difficult.

CELL LINE



v
m

Taken from a tumor tissue & culture as a single cell type.

Normal cells underwent a Genetic change to be tumor cell.

They are m
aster cell
that generate other differentiated cell types.

CELL STRAIN

If a sub population of a cell line is positively selected from the culture by cloning or some other method, this cell line becomes cell strain. A cell strain often acquires additional genetic changes subsequent to the initiation of the parent line

TYPES OF CELL LINES

- Finite cell line
- Continuous cell line

FINITE CELL LINE

- Normal cells are usually divide only an limited number of times before losing their ability to proliferate which is genetically determined event known as **senescence** these lines are known as **finite.**
- The cells normally divide **20 to 100 times** (i.e. 20-100 population doubling) before extinction. The actual number of doubling depends on the species , cell lineage, differences, culture conditions etc.
- The human cells generally divide **50-100 times,** while murine cells divide **30-50 times** before dying.

CONTINUOUS CELL LINE

- When finite cells undergo transformation and acquires the ability to divide indefinitely, it becomes a continuous cell line.
- The Continuous cell lines are transformed, immortal and tumorigenic . The transformed cells for continuous cell lines may be obtained from normal primary cell cultures by treating with chemicals

COMPARISON OF PROPERTIES OF FINITE AND CONTINUOUS CELL LINES

Features	Finite	Continuous
Ploidy	Diploid	Heteroploid
Transformation	Normal	Transformed
Anchorage dependent	Yes	No
density limitation of growth	Yeas	No
Mode of growth	Monolayer	Monolayer with suspension
maintenance	Cyclic	Steady state

Serum requirement	High	Low
Cloning efficiency	Low	High
Markers	Tissue specific	Chromosomal,enzymatic
Virus susceptibility, differentiation	May be retained	Often lost
Growth rate	Slow (24-96 hr)	Rapid (12-24 hr)
Yield	Low	High
Control features	Generatrion number	Strain characteristics

ANIMAL CELL LINES AND PRODUCTS

Cell line	Product
Human tumor	Antigenic factor
Human leucocytes	Interferon
Mouse fibroblasts	Interferon
Human kidney	Urokinase
Duck embryo fluid	Vaccines for rabies & rubella
Human kidney cells	Human protein

APPLICATIONS OF CELL LINE

- Screening of the anti cancerous drug.
- Cell based bioassay.
- To determine the cytotoxicity.
- *In vitro* screening of several drugs.
- production of anti viral vaccines.
- Cell fusion techniques.
- Genetic manipulation.
- Study of the effects of toxins & pollutants using cell lines.
- Study of function of nerve cells.
- Chromosome analysis of cells derived from womb.