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Animal cell science & Technology

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LT 2. Basic Techniques of Mammalian cell culture

Outline

1. Primary Culture
2. Secondary culture
3. Established cell line culture
4. Disaggregation of Tissue
5. Initiation of primary culture
6. Characteristics of primary culture
7. Application of primary culture

What is primary culture?

➢Primary culture refers to culturing of cells in artificial environment by using tissue explants directly isolated from animal/mammalian source.

•A *primary culture* starts with the biopsy (~1 cm^3) from tissue or organ via dissection.

•The tissue or seeded cells grows until they occupy all of the available substrate (i.e., reach **confluence**)

What is secondary culture?

➢The culture formed after sub-culturing of primary culture.

•Sub-culture refers to transfer of certain volume of primary culture in fresh growth medium for continued growth.

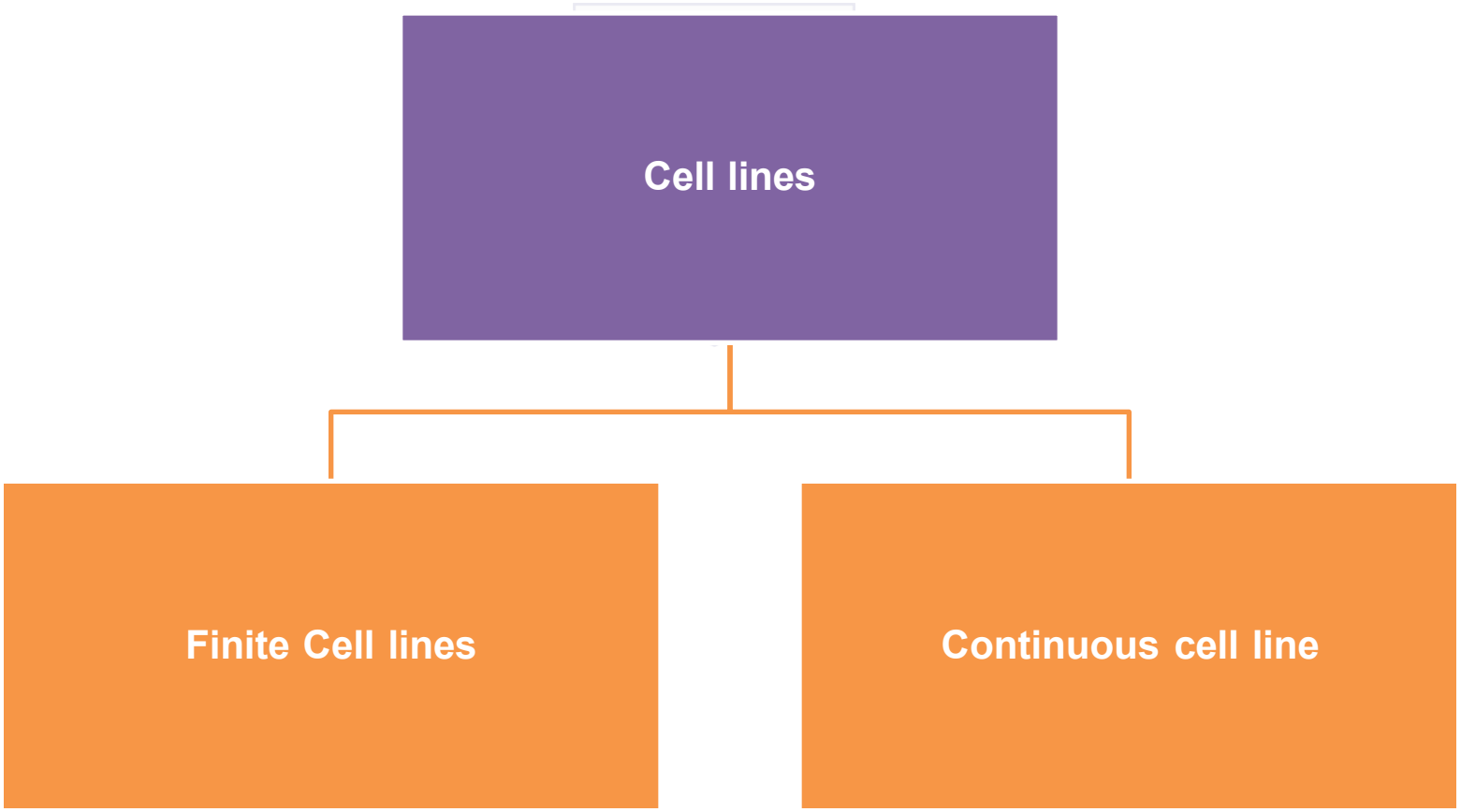
•When the primary culture cells are overgrown and occupied all available substrate it needs to be sub cultured.

Cell line & Cell strain

Cell line:

➢The very first sub culture of primary culture is known as cell line or sub clone.

•They have been continuously passaged **over a long period of time** and have acquired homogenous genotypic and phenotypic characteristics.



Finite cell lines. Continuous cell line, Established cell line

Finite Cell line:

➢Finite cell line have limited cell division capacity.

•A finite cell line has been sub-cultured for 20-80 passages after which they senesce

•The maximum number of times a finite cell divide before losing ability to divide is known as **Hayflick limit**

Continuous cell line :

➢Cells having indefinite cell division capacity and can be maintained indefinitely in cell culture.

•Primary cells undergo transformation leading to loss of cell cycle regulation and control, converts normal cells into cells having unlimited division capacity just like tumourous cells.

Established cell line:

➢A cell line which has achieved ability proliferate indefinitely in an suitable artificial condition and medium is called as established cell line.

•Cells of established cell lines have escaped the hayflick limit and have become immortalized.

Method of creating established cell line & examples

➢Modification of existing cell line using gene editing tools such as CRISPR/ Cas9

•A gene called human telomerase reverse transcriptase (hTERT) can impart immortality to human cell lines.

•hTERT technique (1999) can yield cells that behave like primary cultures but propagate like immortalized lines

➢Immortalization through viral infection. For example, the large T antigen from SV40 virus, or the E6 and E7 oncogenes from human papilloma virus, can quickly turn a primary cell culture into an immortalized line .

•viral oncogenes essentially turn cells into a tumor, they tend to change the cells' characteristics

The above two methods are suitable for establishing non-cancer type normal cell line.

Cell lines	Origin
Vero cell line	kidney of an African green monkey
HeLa cell line	cervical cancer cells (human cervix)
Chinese hamster Ovary (CHO)	epithelial cell line derived from the ovary of the Chinese hamster
HEK 293 cells	Human Kidney

Cell strain

Cell strain:

cells derived from a primary culture or a single *cell* (clone) and possessing a specific feature such as a marker chromosome, antigen, or resistance to a virus. (Medical Dictionary).

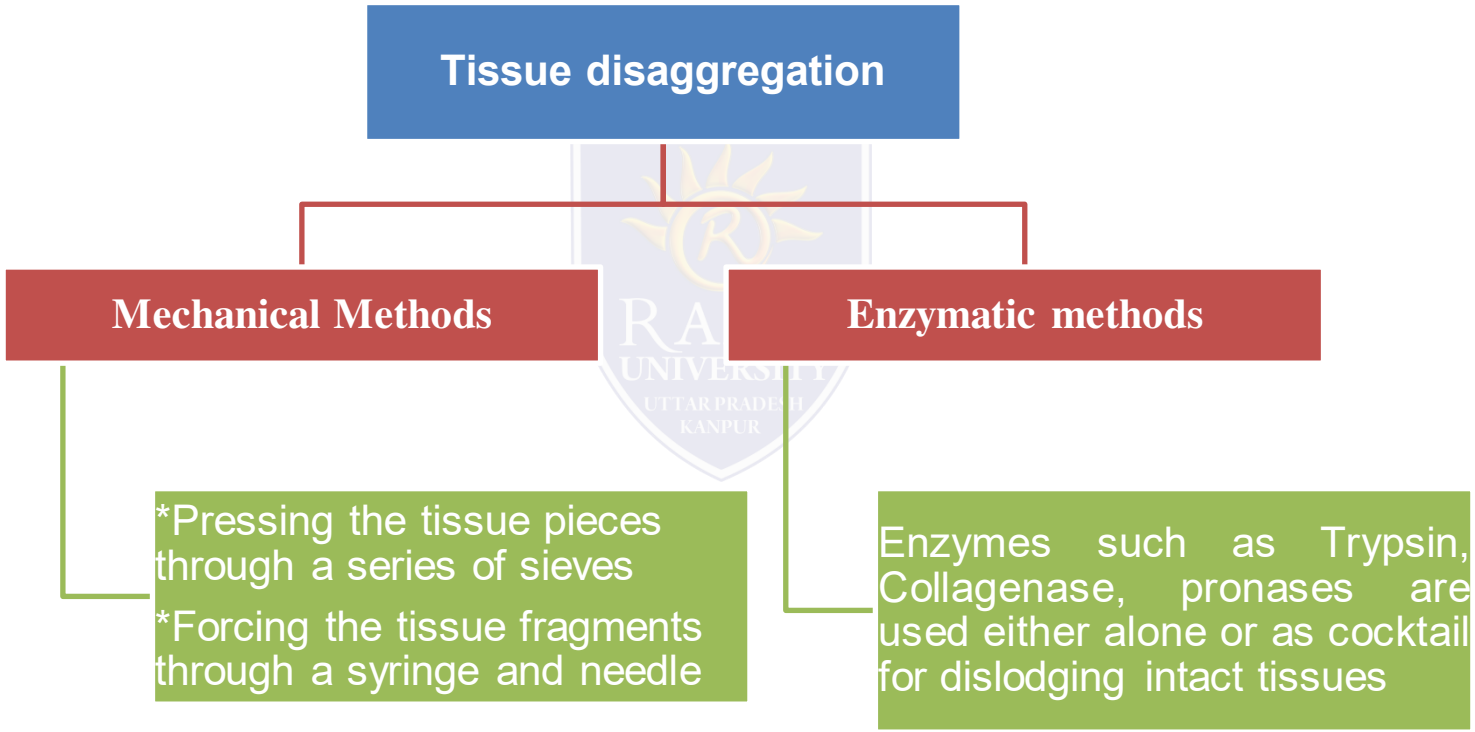
•The specific cells are selected from primary culture by cloning or some other methods. These specific cells give rise to cell strain.

•For example, non-immortalized cells from a cell line can be extracted, alter them with the help of chemicals or a genetically modified virus, and watch them mutate or grow until senescence makes it impossible for them to divide any longer.

Disaggregation of tissues

The whole purpose of disaggregation of excised tissues is to reduce size, remove cell to cell connections and attachments and to convert cluster of cells into single cells.

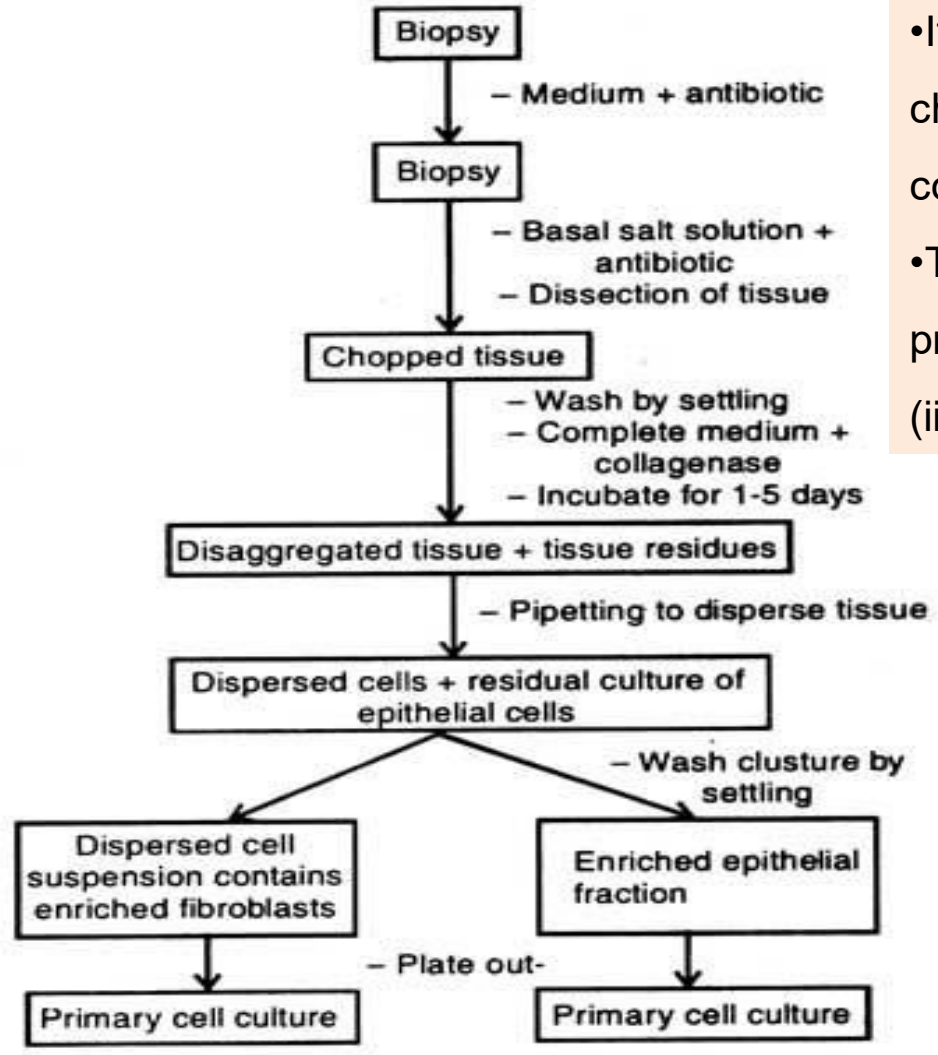
Methods of Tissue disaggregation



•Mechanical methods are simple, inexpensive but it is harsh and has low cell yields.

•Enzymatic methods are tedious, expensive but is mild and has high cell yields.

Process flow for disaggregation of tissue using enzymatic method

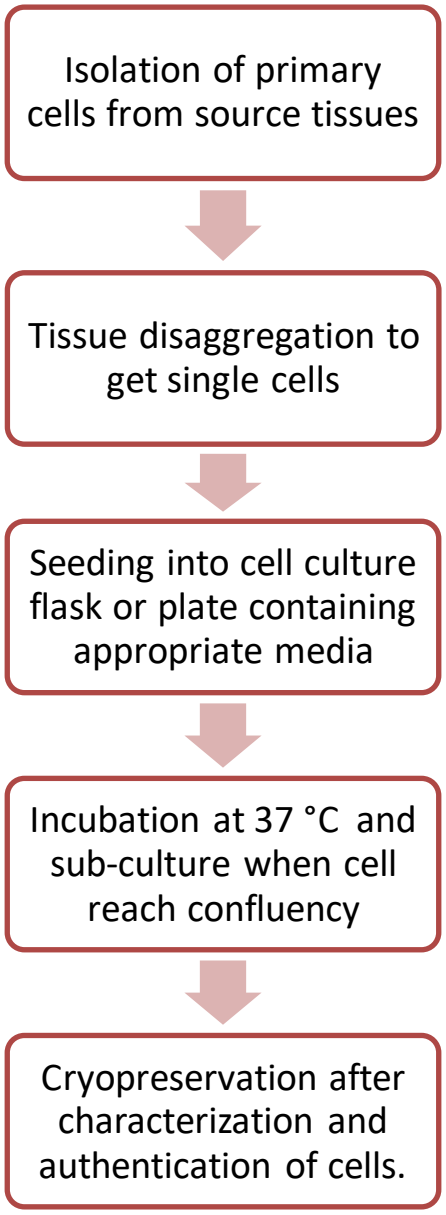


- It can be seen here that tissues were isolated, chopped to reduce size and incubated with collagenase enzymes to yield Single cells.
- Trypsin is also used routinely in two variant process: (i) Cold trypsinization and,

(ii) Hot Trypsinization

Adapted without modification from:
https://biocyclopedia.com/index/biotechnology/animal_biotechnology/animal_cell_tissue_and_organ_culture/biotech_isolation_animal_material.php

Initiation of primary culture



Conditions of primary culture

- Stringent and extreme sterility is required.
- Temperature and pH is dependent upon types of cultured cells. For e.g. mammalian cells are incubated at 37 °C, pH 7.2-7.4; 5-10 % CO_2
- Primary cells can be grown as monolayer as adherent cells and suspension culture as adherent independent cells.
- Adherent cells can be grown on flat surface tissue culture dishes such as single and double coverslip method
- Cells in suspension culture grows floating in cell culture flask. E.g. Flask culture method.
- Hanging drop method of primary culture initiation is used for culturing 3D architecture of cells.

Characteristics of primary culture

•Primary cells have a finite lifespan i.e they can undergo cell division for limited number of time.

•Primary cells retain the true characteristics of original tissues from which they were isolated i.e. they are genetically identical to tissue of source animal.

•Primary cell cultures are harsh, requiring optimized growth conditions, including the addition of specific cytokines and growth factors for propagation in serum-free or low-serum growth media, which is absolutely different from immortalized cell lines.

•The *cultures are* initially *heterogeneous* (represents a mixture of *cell* types present in the tissue)

•They have minimal chromosomal aberrations.

Application of primary cell culture

3D Cell Culture: These cells can act as a model system to study cell biology and biochemistry, to study the interaction between cell and disease-causing agents (like bacteria, virus), to study the effect of drugs, to study the process of aging, to study cell signaling and metabolic regulations.

Cancer Research: Primary cells can be exposed to radiation, chemicals and viruses to make them cancerous. The side effects of cancer treatments (chemotherapy and irradiation) on normal cells can also be studied in this context.

Virology: Detection, isolation, growth and development cycles of viruses can be studied. Primary cells are also useful to study the mode of infection.

Drug Screening and Toxicity Testing: Primary cell cultures are used to study the cytotoxicity of new drugs (to study the effect and safe dosage) and/or drug carriers (nanoparticles). They are also used to determine the maximum permissible dosage of new drugs.

Vaccine Production: Primary animal cells are used in the production of viruses and these viruses are used to produce vaccines (such as vaccines, for deadly diseases like polio, rabies, chicken pox, measles and hepatitis B are produced using animal cell culture) thus avoiding the use of animal models.

Tissue or Organ Replacement: Primary cell cultures can be used as replacement tissue or organs

Stem Cell Therapy: Stem cells isolated from bone marrow, blood or embryo involve primary cell culture. This is an area that is being explored to design therapies for genetic disorders, spinal cord injuries, degenerative diseases and cancer.

References & Further Reading

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