

GROWTH OF ANIMAL CELLS IN CULTURE



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INTRODUCTION

- ❖ An important aspect of any biotechnological processes is the culture of animal cells in artificial media.
- ❖ Cultured animal cells are used in recombinant DNA technology, genetic manipulations and in a variety of industrial processes with economic potential.
- ❖ In production of vaccines, monoclonal antibodies, pharmaceutical drugs, cancer research, etc.



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Major development's in cell culture technology

- ❖ First development was the use of antibiotics which inhibits the growth of contaminants.
- ❖ Second was the use of trypsin to remove adherent cells to subculture further from the culture vessel.
- ❖ Third was the use of chemically defined culture medium.



Why is cell culture used for?



Areas where cell culture technology is currently playing a major role.

- **Model systems**

For studying basic cell biology, interactions between disease causing agents and cells, effects of drugs on cells, process and triggering of aging & nutritional studies.

- **Toxicity testing**

Study the effects of new drugs.

- **Cancer research**

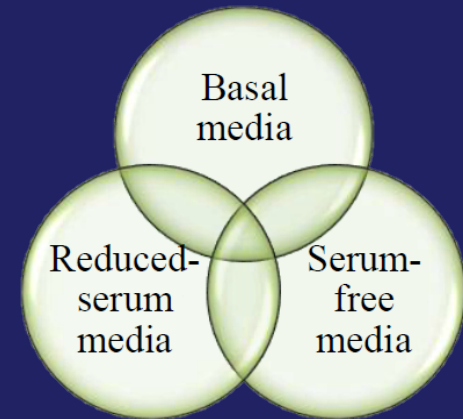
Study the function of various chemicals, virus & radiation to convert normal cultured cells to cancerous cells.

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Cell culture media and reagents

- ❖ The culture medium is the Combination of ingredients which support the cell growth by providing all the essential nutrients , growth factors, and hormones for cell growth, as well as regulating the pH and the osmolarity of culture.
- ❖ The three basic classes of media are: (differ in their requirement for supplementation with serum.)
- ❖ The choice of culture media is dependent on the requirements of cells being cultured.



Basal (Basic) Media :

- Basal Medium is a defined medium that contains essential and nonessential amino acids, vitamins, inorganic salts, organic compounds, and trace elements, but does not contain the Growth Supplements necessary for cell growth.
- Balanced salt solutions (BSS) e.g. phosphate-buffered saline (PBS)
- Dulbecco's Modified Eagle's Medium (**DMEM**) and Roswell Park Memorial Institute Medium (**RPMI 1640**) (with or without glutamine)

Reduced-Serum Media :

- Reduced-serum media are basal media formulations enriched with nutrients and animal-derived factors, which reduce the amount of serum that is needed.



Serum-Free Media :

- Serum-free media (SFM) circumvents issues with using animal sera by replacing the serum with appropriate nutritional and hormonal formulations.
- Serum-free media formulations exist for many primary cultures and cell lines, including Chinese Hamster Ovary (CHO), hybridoma cell lines, VERO, MDCK, MDBK cell lines etc.
- One of the major advantages of using serum-free media is the ability to make the medium selective for specific cell types by choosing the appropriate combination

f growth factors.
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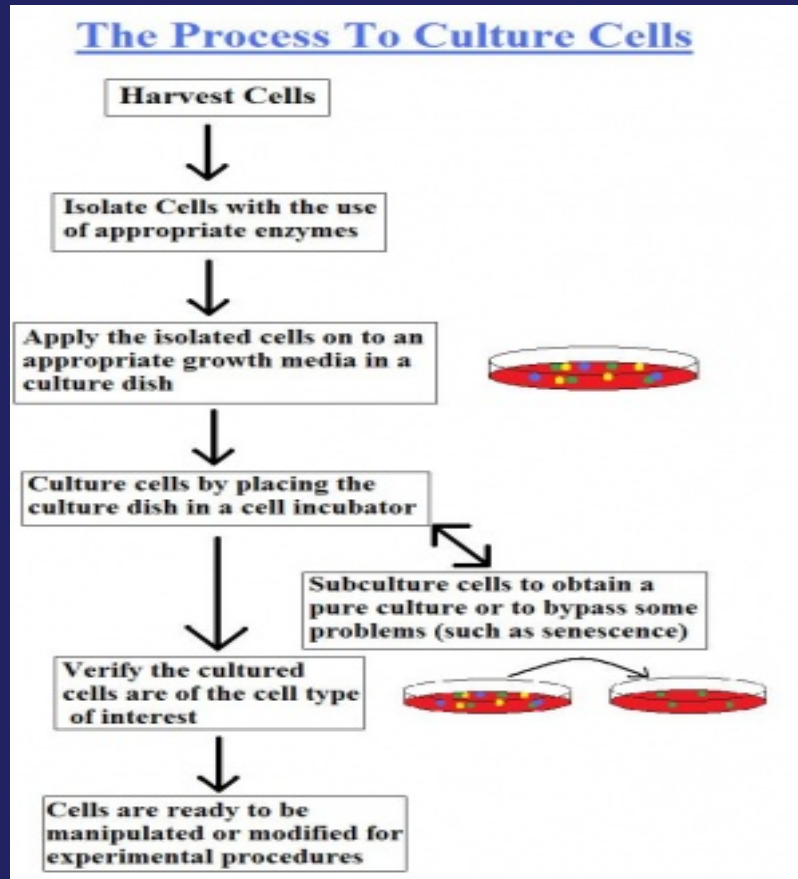


ANIMAL CELL CULTURE

- ❖ Cell culture refers to the process by which cells are grown in a controlled artificial environment. Cells can be maintained in vitro outside of their original body by this process.
- ❖ In a cell culture technique, cells are removed from an animal or a plant, and grown subsequently in a favorable environment. For animal cell culture the cells are taken from the organ of an experimental animal.
- ❖ The cells may be removed directly or by mechanical or enzymatic action.
- ❖ Ex. fibroblast, lymphocytes, cells from cardiac and skeletal tissues, cells from liver, breast, skin, and kidney and different types of tumor cells.



PROCESS OF CELL CULTURE



Types of animal cell culture

Based on the number of cell division, cell culture can be classified as

1. primary cell culture
2. cell lines

Cell lines can undergo either finite or infinite cell divisions.



ANIMAL CELL CULTURE CLASSIFICATION

A. Primary cell culture

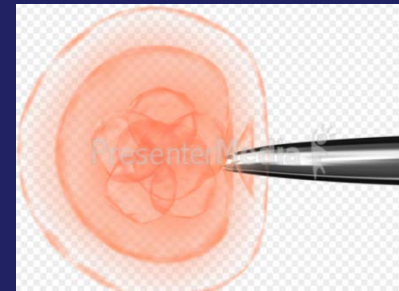
Depending on their origin, primary cells grow either as an adherent monolayer or in a suspension:

- ✓ Adherent cells
- ✓ Suspension cells
- ✓ Confluent culture and the necessity of sub-culture

B. Secondary cell culture and cell line

On the basis of the life span of culture, the cell lines are categorized into two types:

- ✓ Finite cell lines
- ✓ Continuous cell lines



A. Primary cell culture

- This is the cell culture obtained straight from the cells of a host tissue.
- The cells dissociated from the parental tissue are grown on a suitable container and the culture thus obtained is called primary cell culture.
- Such culture comprises mostly heterogeneous cells and most of the cells divide only for a limited time. However, these cells are much similar to their parents.



Adherent cells

- ❑ These cells are anchorage dependent and propagate as a monolayer.
- ❑ These cells need to be attached to a solid or semi-solid substrate for proliferation.
- ❑ These adhere to the culture vessel with the use of an extracellular matrix which is generally derived from tissues of organs that are immobile and embedded in a network of connective tissue.



Ex- Fibroblasts and epithelial cells

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Suspension cells

- ✓ Suspension cells do not attach to the surface of the culture vessels. These cells are also called anchorage independent or non-adherent cells which can be grown floating in the culture medium.
- ✓ Hematopoietic stem cells (derived from blood, spleen and bone marrow) and tumor cells can be grown in suspension.
- ✓ These cells grow much faster which do not require the frequent replacement of the medium and can be easily maintained.
- ✓ These are of homogeneous types and enzyme treatment is not required for the dissociation of cells; similarly these cultures have short lag period.



Confluent culture and the necessity of sub-culture

- ❖ After the cells are isolated from the tissue and proliferated under the appropriate conditions, they occupy all of the available substrate i.e. reach confluence.
- ❖ For a few days it can become too crowded for their container and this can be detrimental to their growth, generally leading to cell death if left for long time. The cells thus have to be subculture i.e. a portion of cells is transferred to a new vessel with fresh growth medium which provides more space and nutrients for continual growth of both portions of cells.

❖ Hence subculture keeps cells in healthy and in growing state.

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A passage number

- It refers specifically to how many times a cell line has been sub-cultured.
- In contrast with the population doubling level in that the specific number of cells involved is not relevant.
- It simply gives a general indication of how old the cells may be for various assays.



B. Secondary cell culture and cell line

- ✓ When a primary culture is sub-cultured, it is known as secondary culture or cell line or sub-clone.
- ✓ The process involves removing the growth media and disassociating the adhered cells (usually enzymatically).
- ✓ Sub-culturing of primary cells to different divisions leads to the generation of cell lines. During the passage, cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population.
- ✓ However, as they are sub-cultured serially, they become different from the original cell.



Finite cell lines

- The cell lines which go through a limited number of cell division having a limited life span are known as finite cell lines.
- The cells passage several times and then lose their ability to proliferate, which is a genetically determined event known as **senescence**.
- Cell lines derived from primary cultures of normal cells are finite cell lines.



Continuous cell lines

- When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a continuous cell line.
- Such transformation/mutation can occur spontaneously or can be chemically or virally induced or from the establishment of cell cultures from malignant tissue.
- These cells are less adherent, fast growing, less fastidious in their nutritional requirements, able to grow up to higher cell density and different in phenotypes from the original tissue.
- Such cells grow more in suspension.
- They also have a tendency to grow on top of each other in multilayers on culture vessel surfaces.

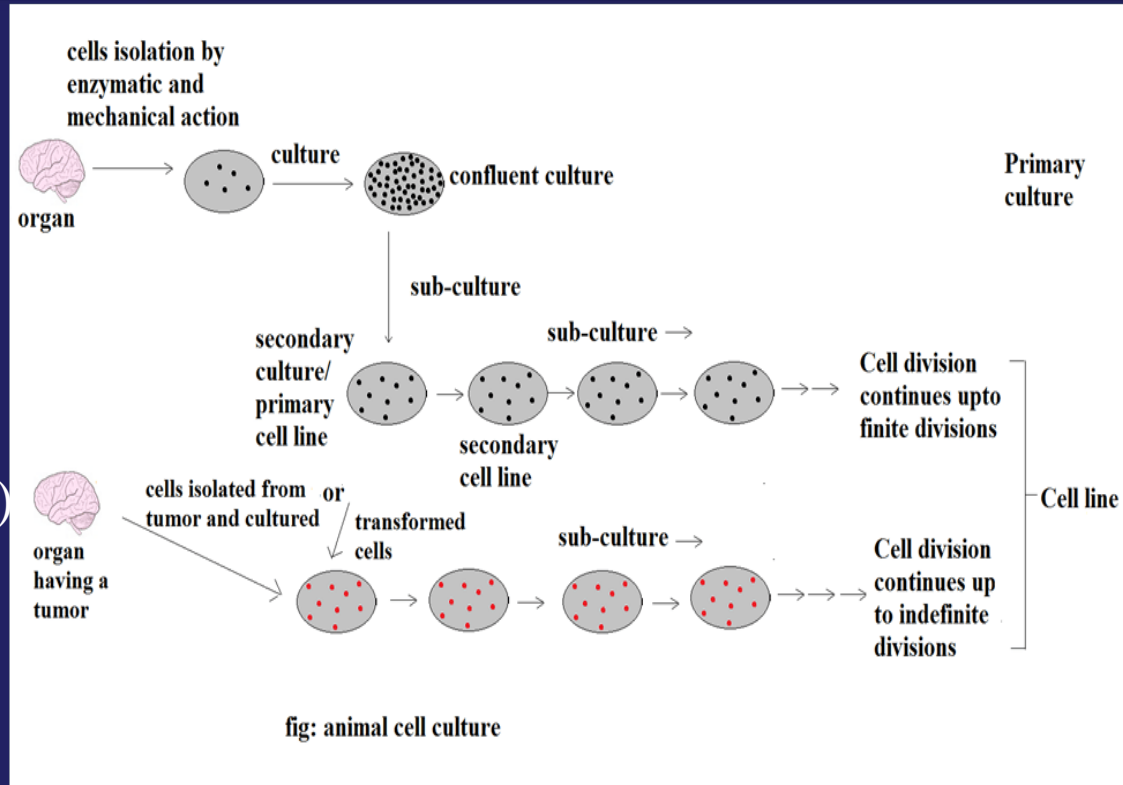


ANIMAL CELL CULTURE

Common cell lines

Human cell lines:

- ❖ MCF-7 (breast cancer)
- ❖ HL 60 (Leukemia)
- ❖ HeLa (Human cervical cancer cells)



METHODS

❖ Growth Requirements

❖ Process to obtain primary cell culture

❖ Aseptic techniques

❖ Cryopreservation



GROWTH REQUIREMENTS

The culture media generally include amino acids, vitamins, salts (maintain osmotic pressure), glucose, a bicarbonate buffer system (maintains a pH between 7.2 and 7.4), growth factors, hormones, O₂ and CO₂.

To obtain best growth, addition of a small amount of blood serum is usually necessary, and several antibiotics, like penicillin and streptomycin are added to prevent bacterial contamination.

Temperature varies on the type of host cell. Most mammalian cells are maintained at 37°C for optimal growth, while cells derived from cold-blooded animals tolerate a wider temperature range (i.e. 15°C to 26°C).



Actively growing cells of log phase should be used which divide rapidly during

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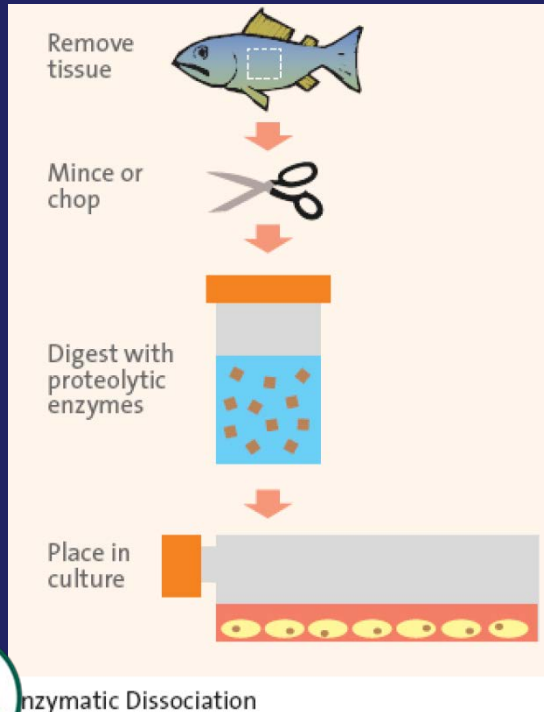
PROCESS TO OBTAIN PRIMARY CELL CULTURE

Primary cell cultures are prepared from fresh tissues. Pieces of tissues from the organ are removed aseptically; which are usually minced with a sharp sterile razor and dissociated by proteolytic enzymes (such as trypsin) that break apart the intercellular cement.

The obtained cell suspension is then washed with a physiological buffer (to remove the proteolytic enzymes used).

The cell suspension is spread out on the bottom of a flat surface, such as a bottle or a Petri dish. This thin layer of cells adhering to the glass or plastic dish is overlaid with a suitable culture medium and is incubated at a suitable temperature.





Corning culture flasks are used for growing anchorage-dependent cells.



ASEPTIC TECHNIQUES

- Bacterial infections, like Mycoplasma and fungal infections commonly occur in cell culture creating a problem to identify and eliminate.
- Thus, all cell culture work is done in a sterile environment with proper aseptic techniques.
- Work should be done in laminar flow with constant unidirectional flow of HEPA filtered air over the work area.
- All the material, solutions and the whole atmosphere should be contamination-free.



CRYOPRESERVATION

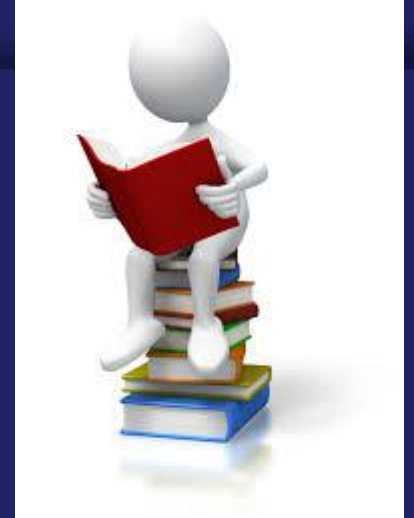
- If a surplus of cells is available from sub-culturing, they should be treated with the appropriate protective agent (e.g., DMSO or glycerol) and stored at temperatures below -130°C until they are needed.
- This stores cell stocks and prevents original cell from being lost due to unexpected equipment failure or biological contaminations. It also prevents finite cells from reaching senescence and minimizes risks of changes in long term cultures.
- When thawing the cells, the frozen tube of cells is warmed quickly in warm water, rinsed with medium and serum and then added into culture containers once suspended in the appropriate media.



APPLICATIONS OF ANIMAL CELL CULTURE

The nine applications of animal cell culture are:

- (1) Model Systems
- (2) Toxicity Testing
- (3) Cancer Research
- (4) Virology
- (5) Cell-Based Manufacturing
- (6) Genetic Counselling
- (7) Genetic Engineering
- (8) Gene Therapy and
- (9) Drug Screening and Development



Application # 1. Model Systems

Cell cultures provide a good model system for studying:

- (1) Basic cell biology and biochemistry
- (2) The interactions between disease-causing agents and cells
- (3) The effects of drugs on cells
- (4) The process and triggers for aging
- (5) Nutritional studies



Application # 2. Toxicity Testing

- Cultured cells are widely used alone or in conjunction with animal tests to study the effects of new drugs, cosmetics and chemicals on survival and growth in a wide variety of cell types.
- Especially important are liver- and kidney-derived cell cultures.



Application # 3. Cancer Research

- Since both normal cells and cancer cells can be grown in culture, the basic differences between them can be closely studied.
- In addition, it is possible, by the use of chemicals, viruses and radiation, to convert normal cultured cells to cancer causing cells.
- Cultured cancer cells also serve as a test system to determine suitable drugs and methods for selectively destroying types of cancer.



Application # 4. Virology

- One of the earliest and major uses of cell culture is the replication of viruses in cell cultures (in place of animals) for use in vaccine production.
- Cell cultures are also widely used in the clinical detection and isolation of viruses, as well as basic research into how they grow and infect organisms.



Application # 5. Cell-Based Manufacturing

- Cultured cells can be used to produce many important products, three areas are generating the most interest.
- The first is the large-scale production of viruses for use in vaccine production.
Ex. Vaccines for polio, rabies, chicken pox, hepatitis B and measles.
- The second is the large-scale production of cells that have been genetically engineered to produce proteins that have medicinal or commercial value.
Ex. Monoclonal antibodies, insulin, hormones, etc.
- The third is the use of cells as replacement tissues and organs.
Ex. Artificial skin for use in treating burns and ulcers is the first commercially available product and artificial organs such as pancreas, liver and kidney is underway.



Application # 6. Genetic Counseling

- Amniocentesis, a diagnostic technique that enables remove and culture of fetal cells from pregnant women, has given an important tool for the early diagnosis of fetal disorders.
- These cells can then be examined for abnormalities in their chromosomes and genes using karyotyping, chromosome painting and other molecular techniques.



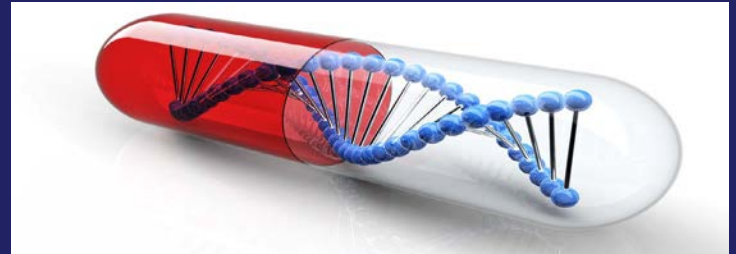
Application # 7. Genetic Engineering

- The ability to transfect or reprogram cultured cells with new genetic material (DNA and genes) has provided a major tool to study the cellular effects of the expression of these genes (new proteins).
- These techniques can also be used to produce these new proteins in large quantity in cultured cells for further study.
- Insect cells are widely used as miniature cells factories to express substantial quantities of proteins that they manufacture after being infected with genetically engineered baculoviruses.



Application # 8. Gene Therapy

- The ability to genetically engineer cells has also led to their use for gene therapy. Cells can be removed from a patient lacking a functional gene and the missing or damaged gene can then be replaced.
- The cells can be grown for a while in culture and then replaced into the patient.
- An alternative approach is to place the missing gene into a viral vector and then “infect” the patient with the virus in the hope that the missing gene will then be expressed in the patient’s cells.



Application # 9. Drug Screening and Development

- Cell-based assays have become increasingly important for the pharmaceutical industry, not just for cytotoxicity testing but also for high throughput screening of compounds that may have potential use as drugs.
- Originally, these cell culture tests were done in 96 well plates, but increasing use is now being made of 384 and 1536 well plates.



**HAPPY TO ANSWER IF U
HAVE ANY QUESTION**

THANK YOU



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