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- ❖ Introduction
- ❖ Types of cell culture
- ❖ General procedure for cell cultures
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- ▶ Module 5:- Cell culture techniques
- ▶ Basic equipments used in cell culture lab. Cell culture media, various types of cell culture, general procedure for cell cultures; isolation of cells, subculture, cryopreservation, characterization of cells and their application.

Lecture No. 1/Course Objectives

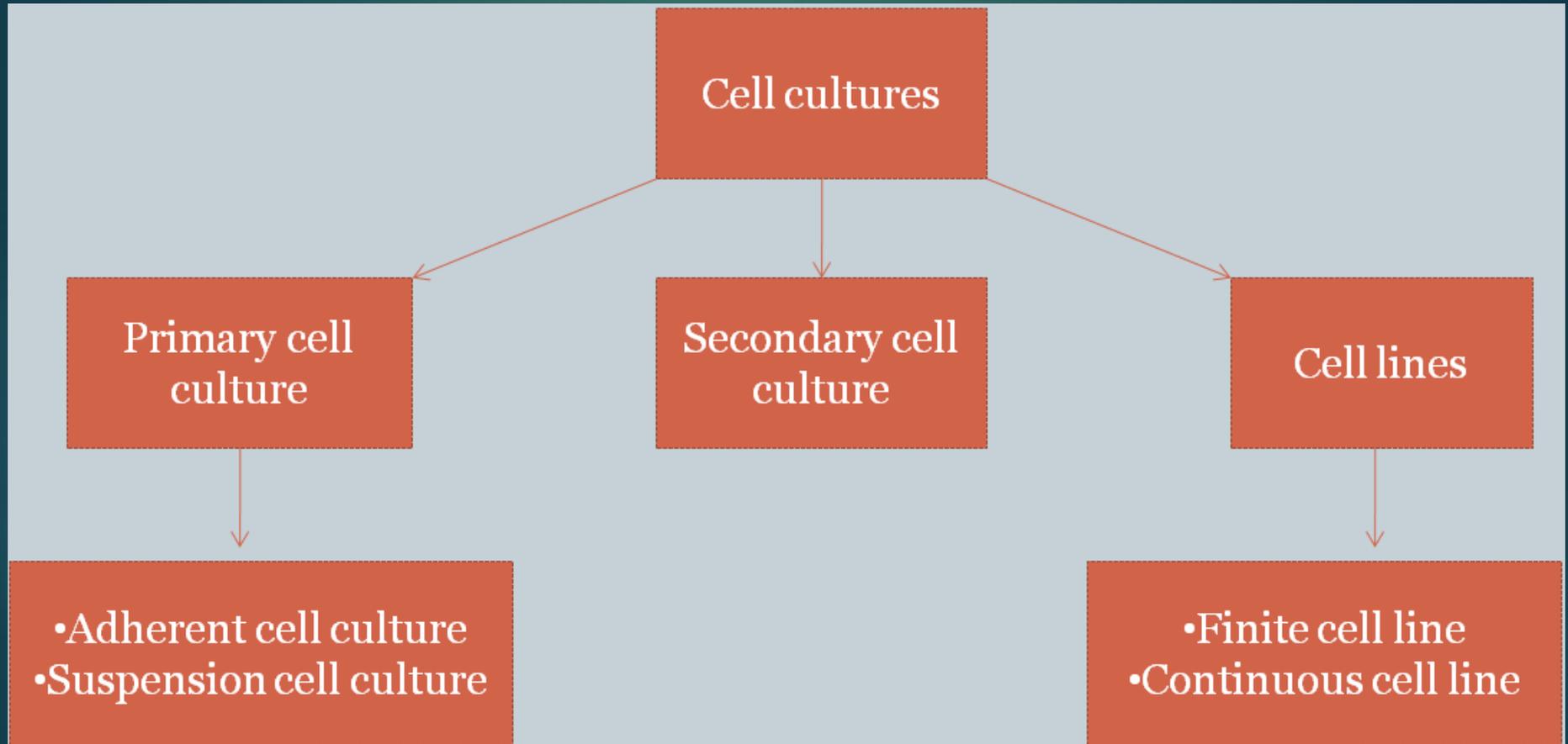
- Understand what are cell culture techniques , general procedures.
- Use of these techniques in pharmaceutical for the production of medicines

Introduction



- Cell culture is the multifaceted process through which cells are isolated from an animal or plant and their subsequent growth under controlled artificial conditions, generally outside their natural environment. In this procedure cells are directly isolated from body or disaggregated by enzymatic or mechanical procedure.
- Cell culture was first successfully undertaken by Ross Harrison in 1907
- Roux in 1885 for the first time maintained embryonic chick cells in a cell culture

Types of cell cultures



Types of cell cultures

Primary culture :- refers to the stage (primary step) of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate. **At this stage, the cells have to be subcultured** by transferring them to a new vessel with fresh growth medium to provide more room for continued growth.

- Primary culture contains a very heterogeneous population of cells.
- Primary cells have a finite life span.
- Sub culturing of primary cells leads to the generation of cell lines.

Types of cell cultures

Cell Line:-After the first subculture, the primary culture becomes known as a **cell line or subclone**.

- Cell lines derived from primary cultures have a limited life span.
- Cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population.

Types of cell cultures

Cell Strain:- Subpopulation of a cell line is positively selected from the culture by cloning or some other method, this cell line becomes a **cell strain**.

- A cell strain often acquires additional genetic changes subsequent to the initiation of the parent line.
- These cells have a finite lifespan of 40-60 divisions in vitro.
- They are useful in vaccine production.

General procedure for cell cultures

Aseptic technique



Preparation of glassware

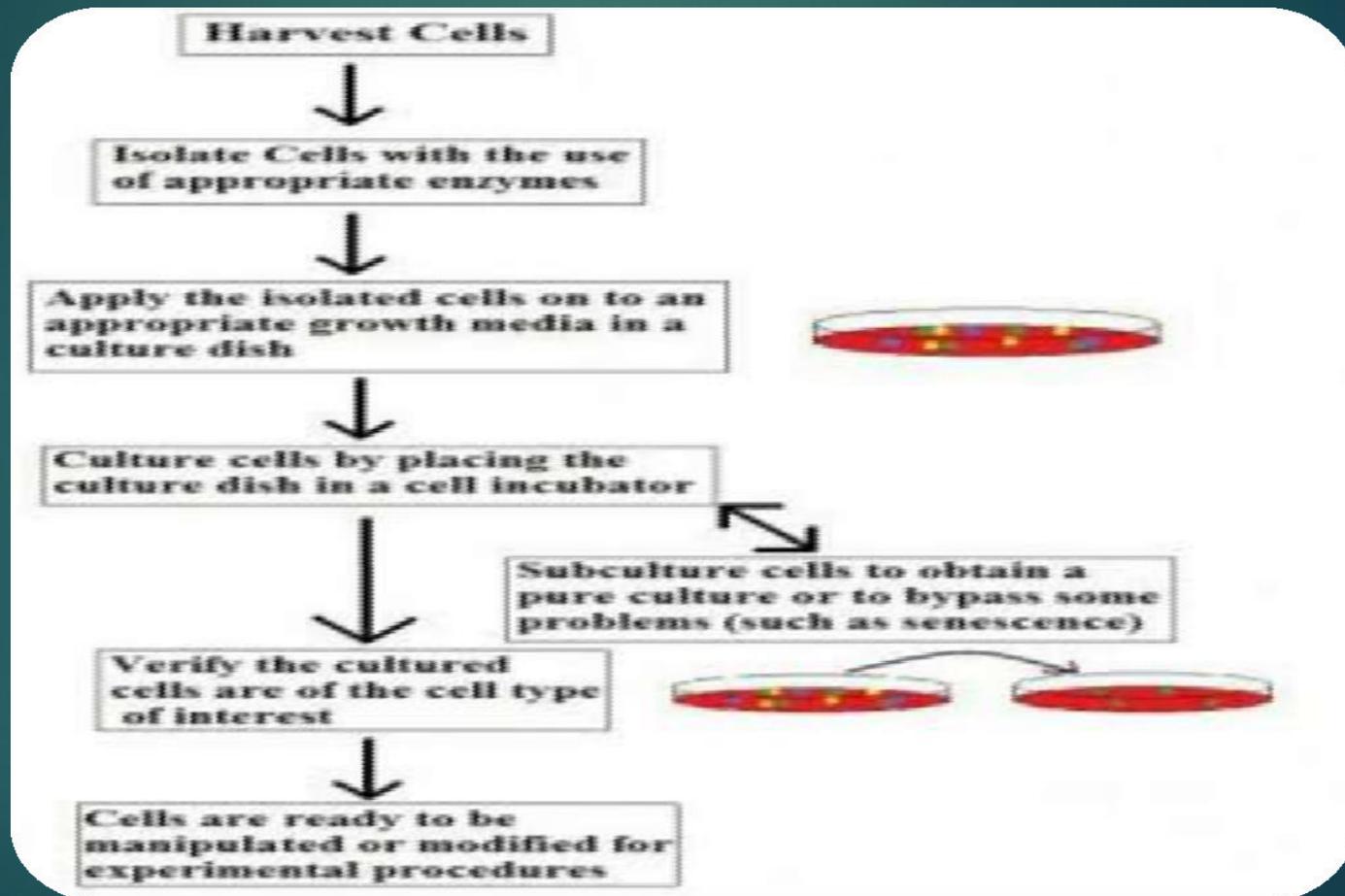


Selection of cell culture systems



Preparation of cell culture systems

General procedure for cell cultures



General procedure for cell cultures

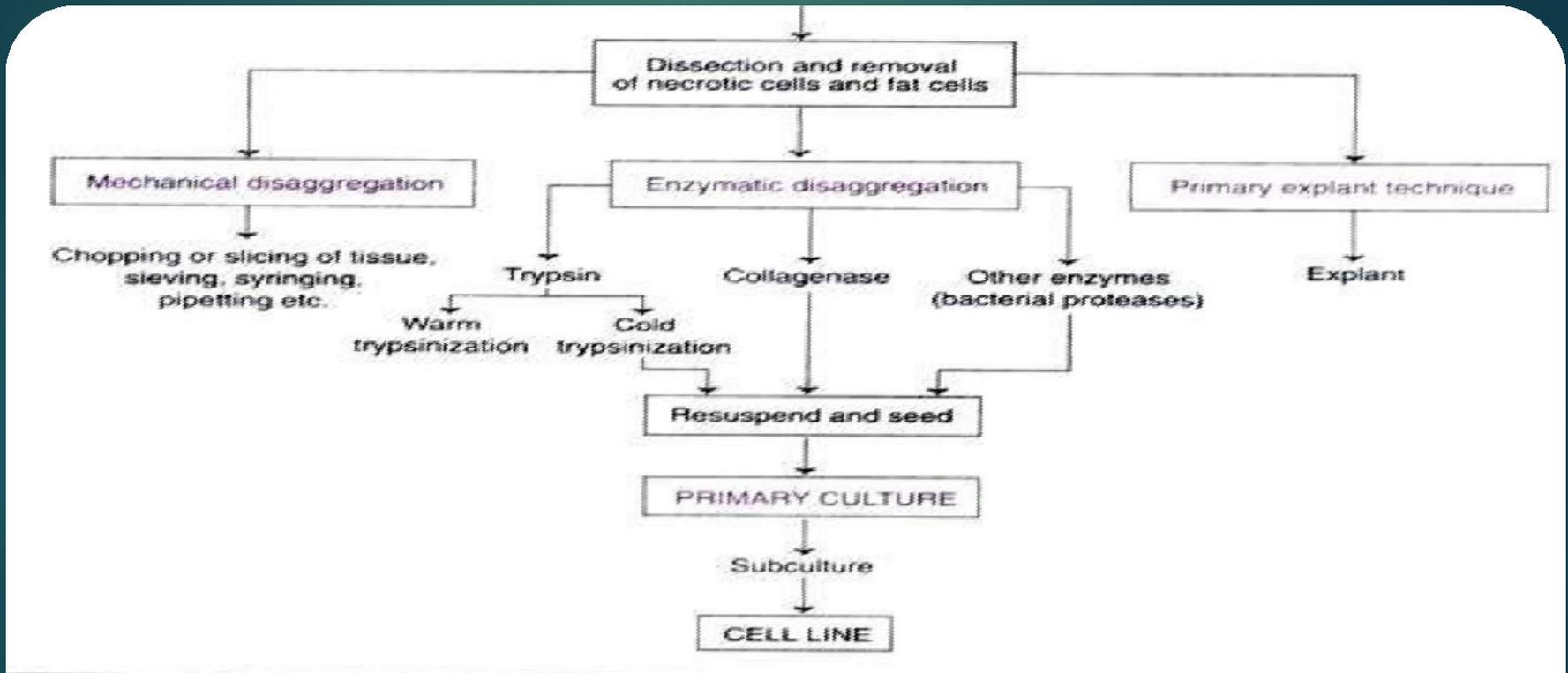


Fig. 36.1 : Different techniques used for primary culture.

Application of cell culture

•**Model System:-** Cell culture are used as model system to study basic 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 and also it is used to study triggers for ageing.

•**Cancer Research-** The basic difference between normal cell and cancer cell can be studied using animal cell culture technique, as both cells can be cultured in laboratory. Normal cells can be converted into cancer cells by using radiation, chemicals and viruses. Thus, the mechanism and cause of cancer can be studied. Cell culture can be used to determine the effective drugs for selectively destroy only cancer cells

Application of cell culture

- **Virology**:- Animal cell cultures are used to replicate the viruses instead of animals for the production of vaccine. Cell culture can also be used to detect and isolate viruses, and also to study growth and development cycle of viruses. It is also used to study the mode of infection.
- **Toxicity Testing**:- Animal cell culture is used to study the effects of new drugs, cosmetics and chemicals on survival and growth of a number of types of cells. Especially liver and kidney cells. Cultured animal cells are also used to determine the maximum permissible dosage of new drugs.

Application of cell culture

- **Vaccine Production:**- Cultured animal cells are used in the production of viruses and these viruses are used to produce vaccines. For example vaccines for deadly diseases like polio, rabies, chicken pox, measles and hepatitis B are produced using animal cell culture.
- **Genetically Engineered Protein:**- Animal cell cultures are used to produce commercially important genetically engineered proteins such as monoclonal antibodies, insulin, hormones, and much more.

Application of cell culture

• **Replacement Tissue or Organ:-** Animal cell culture can be used as replacement tissue or organs. For example artificial skin can be produced using this technique to treat patients with burns and ulcers. However research is going on artificial organ culture such as liver, kidney and pancreas. Organ culture techniques and research are being conducted on both embryonic and adult stem cell culture. These cells have the capacity to differentiate into many different types of cells and organs. It is believed that by learning to control the development and differentiation of these cells may be used to treat variety of medical conditions.

Application of cell culture

- **Genetic Counseling:-** Fetal cell culture extracted from pregnant women can be used to study or examine the abnormalities of chromosomes, genes using karyotyping, and these findings can be used in early detection of fetal disorders.
- **Genetic Engineering:-** Cultured animal cells can be used to introduce new genetic material like DNA or RNA into the cell. These can be used to study the expression of new genes and its effect on the health of the cell. Insect cells are used to produce commercially important proteins by infecting them with genetically altered baculoviruses.

Application of cell culture

Gene Therapy:- Cultured animal cells can be genetically altered and can be used in gene therapy technique. First cells are removed from the patient lacking a functional gene or missing a functional gene. These genes are replaced by functional genes and altered cells are culture and grown in laboratory condition. Then these altered cells are introduced into the patient. Another method is by using viral vector, functional gene is inserted into the genome of viral vector and then they are allowed to infect the patient, in the hope that the missing gene will be expressed with the help of the viral vector.

Application of cell culture

- **Drug Screening and Development:-** Animal cell cultures are used to study the cytotoxicity of new drug. This is also used to find out the effective and safe dosage of new drugs. Now these tests are being conducted in 384 and 1536 well plates. Cell-based assay plays an important role in pharmaceutical industry.