Tissue Culture

- Tissue culture is the term used for “the process of growing cells artificially in the laboratory”
- Tissue culture involves both plant and animal cells
- Tissue culture produces clones, in which all product cells have the same genotype (unless affected by mutation during culture)

History

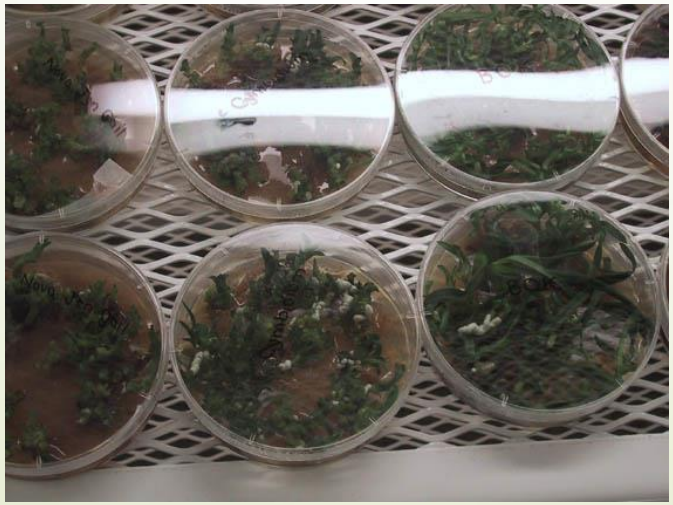
Haberlandt

Carrel

- Tissue culture had its origins at the beginning of the 20th century with the work of Gottleib Haberlandt (plants) and Alexis Carrel (animals)

The First artificial medium

- The first commercial use of plant clonal propagation on artificial media was in the germination and growth of orchid plants, in the 1920's
- In the 1950's and 60's there was a great deal of research, but it was only after the development of a reliable artificial medium (**Murashige & Skoog, 1962**) that plant tissue culture really 'took off' commercially



Critical requirements of Plant and Animal Tissue culture

- Appropriate tissue (some tissues culture better than others)
- A suitable growth medium containing energy sources and inorganic salts to supply cell growth needs. This can be liquid or semisolid
- Aseptic (sterile) conditions, as microorganisms grow much more quickly than plant and animal tissue and can over run a culture

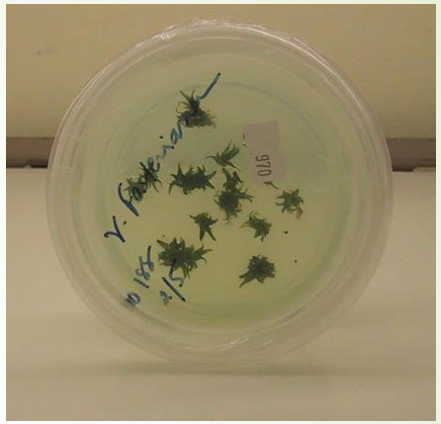
- Growth regulators - in plants, both auxins & cytokinins. In animals, this is not as well defined and the growth substances are provided in serum from the cell types of interest
- Frequent subculturing to ensure adequate nutrition and to avoid the build up of waste metabolites

Plant Tissue Culture

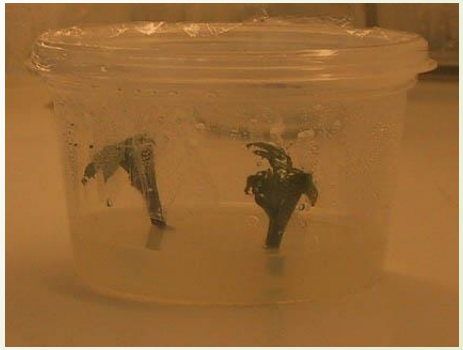
Steps involved in Plant Tissue Culture

- Selection of the plant tissue (explant) from a healthy vigorous 'mother plant' - this is often the apical bud, but can be other tissue as well.
- This tissue must be sterilized to remove microbial contaminants.

- Establishment of the explant in a culture medium. The medium sustains the plant cells and encourages cell division. It can be solid or liquid



- Each plant species (and sometimes the variety within a species) has particular medium requirements that must be established by trial and error

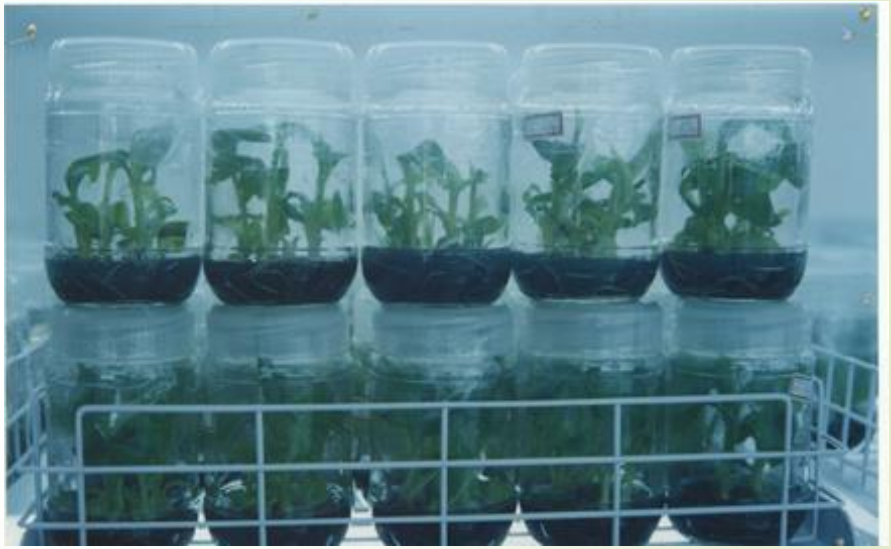


Dividing shoots

Growing in warmth and good light

- Multiplication- The explant gives rise to a callus (a mass of loosely arranged cells) which is manipulated by varying sugar concentrations and the low auxin : cytokinin ratios to form multiple shoots
- The callus may be subdivided a number of times

- Root formation - The shoots are transferred to a growth medium with relatively higher auxin: cytokinin ratios



Young banana plants

- The rooted shoots are potted up (deflasked) and ‘hardened off’ by gradually decreasing the humidity
- This is necessary as many young tissue culture plants have no waxy cuticle to prevent water loss

Applications:

- A single explant can be multiplied into several thousand plants in less than a year - this allows fast commercial propagation of new cultivars.
- Taking an explant does not usually destroy the mother plant, so rare and endangered plants can be cloned safely.
- Once established, a plant tissue culture line can give a continuous supply of young plants throughout the year

- In plants prone to virus diseases, virus free explants (new meristem tissue is usually virus free) can be cultivated to provide virus free plants.
- Plant ‘tissue banks’ can be frozen, then regenerated through tissue culture.
- Plant cultures in approved media are easier to export than are soil-grown plants, as they are pathogen free and take up little space.
- Tissue culture allows fast selection for crop improvement - explants are chosen from superior plants, then cloned.
- Tissue culture clones are ‘true to type’ as compared with seedlings, which show greater variability.

Animal Tissue Culture

Animal tissue/cell culture - differences from plant tissue culture

- Animal cell lines have limited numbers of cell cycles before they begin to degrade
- Animal cells need frequent subculturing to remain viable
- Tissue culture media is not as fully defined as that of plants - in addition to inorganic salts, energy sources, amino acids, vitamins, etc., they require the addition of serum (bovine serum is very common, but others are used)

Types of cell cultured

Primary cultures

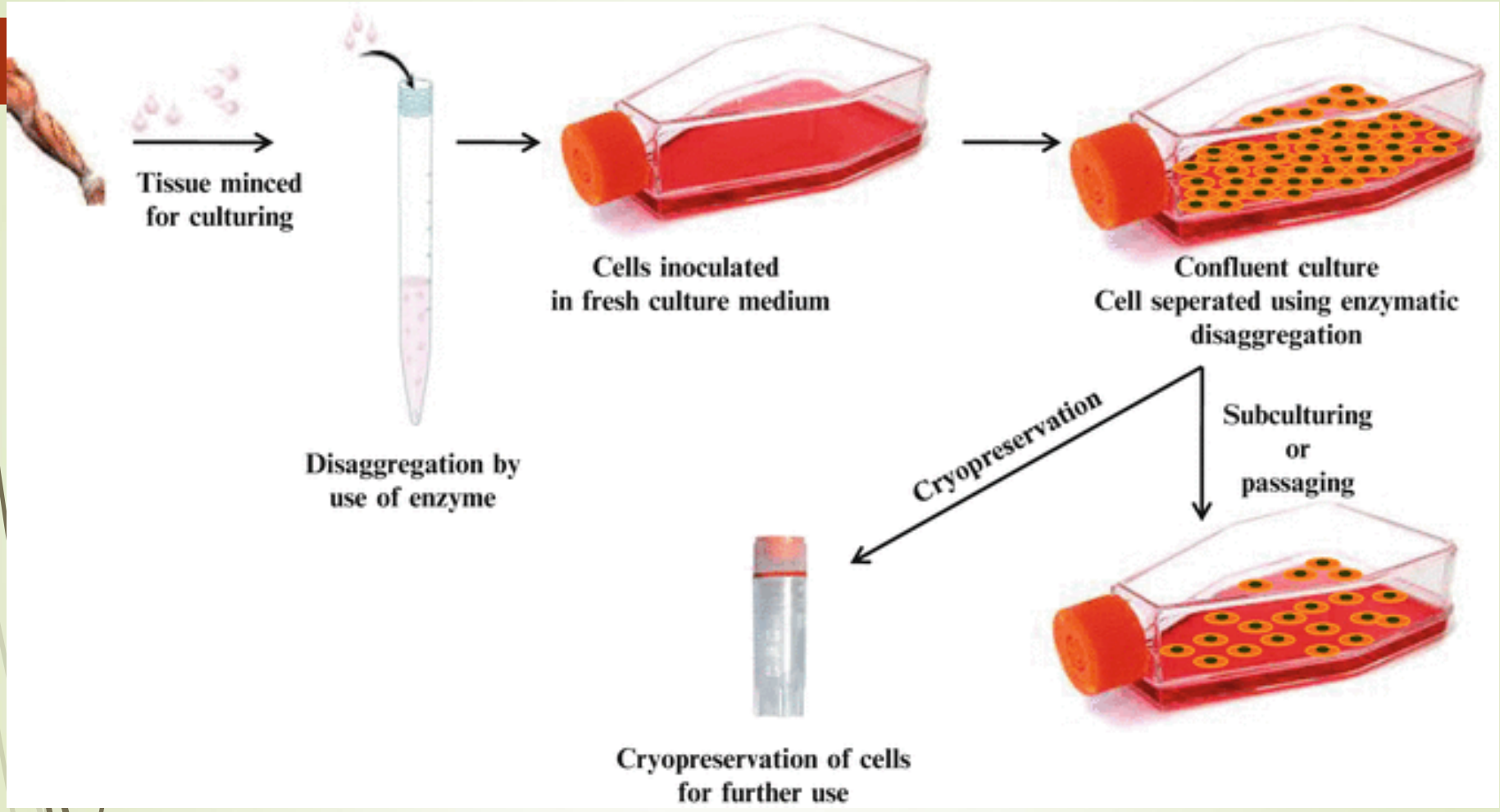
- Derived directly from adult animal tissue or embryo.
- Normal or neoplastic
- Cultured either as tissue explants or single cells.
- Finite life span in vitro.
- Mainly anchorage dependent.

Secondary cultures

- Derived from a primary cell culture.
- Isolated by selection or cloning.
- Becoming a more homogeneous cell population.
- Finite life span in vitro.
- Mainly anchorage dependent.

Continuous cultures

- Derived from a primary or secondary culture
- Immortalized
- Spontaneous genetic mutation
- By transformation vectors (e.g.: viruses &/or plasmids)
- Serially propagated in culture showing an increased growth rate
- Homogeneous cell population
- Loss of anchorage dependency and contact inhibition
- Infinite life span in vitro
- Genetically unstable
- Characteristics of continuous cell lines -smaller, more rounded, less adherent with a higher nucleus /cytoplasm ratio -Fast growth and have aneuploid chromosome number.


Tissue minced
for culturing
Disaggregation by
use of enzyme
Cells inoculated
in fresh culture medium
Confluent culture
Cell seperated using enzymatic
disaggregation
Cryopreservation
Subculturing
or
passaging
Cryopreservation of cells
for further use

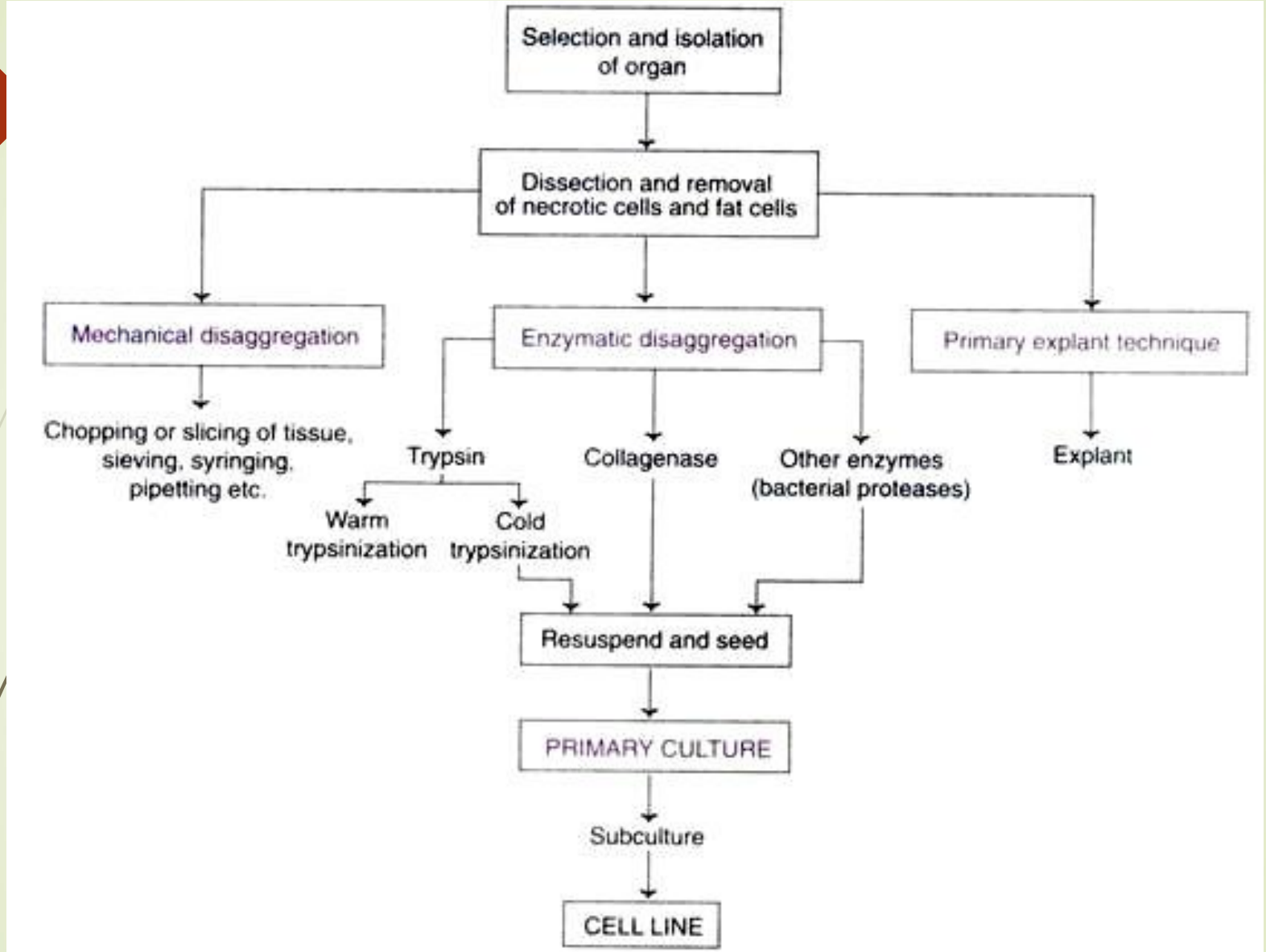


Fig. 36.1 : Different techniques used for primary culture.

- Animal tissue cultures can pose biohazard concerns, and cultures require special inactivation with hypochlorite followed by incineration.

Gloves and labcoat are always worn. The pipettes used are disposable.

MEDIA COMPOSITION

- Most animal cell culture media are generally having following basic components and they are as follows:
- Energy sources: Glucose, Fructose, Amino acids
- Nitrogen sources: Amino acids
- Vitamins: Generally water soluble vitamins B & C.
- Inorganic salts: Na^{+}, K^{+}, Ca^{+2}, Mg^{+2}

- Fat and Fat soluble components: Fatty acids, cholesterols
- Antibiotics
- Growth factors and hormones
- Oxygen and CO_2 concentration.
- The physical environment includes the optimum pH, temperature, osmolality and gaseous environment, supporting surface and protecting the cells from chemical, physical, and mechanical stresses

- CO_2 incubators are used and designed to mimic the environmental conditions of the living cells.
- An inverted microscope is used for visualizing cell cultures invitro.
- For most animal cell cultures low speed centrifuges are needed
- Cryo preservation is storing of cells at very low temperature (-1800C to -196 0C) using liquid nitrogen. DMSO is a cryopreservative molecule which prevents damage to cells.
- Serum is essential for animal cell culture and contains growth factors which promote cell proliferation

SERUM

- Liquid yellowish, clear content left over after fibrin and cells are removed from the blood is known as serum.
- Fetal bovine serum (FBS) is the most commonly applied supplement in animal cell culture media. Normal growth media often contain 2-10% of serum.
- It provides the basic nutrients for cells
- Provides several hormones
- Contains several growth factor
- And it also acts as a buffer.

APPLICATIONS

- Production of antiviral vaccines
- Cancer research, which requires the study of uncontrolled cell division in cultures.
- Production of pharmaceutical drugs using cell lines.
- Study the function of the nerve cells.
- Many commercial proteins have been produced by animal cell culture and there medical application is being evaluated. Tissue Plasminogen activator (t-PA) was the first drug that was produced by the mammalian cell culture by using rDNA technology. The recombinant t-PA is safe and effective for dissolving blood clots in patients with heart diseases and thrombotic disorders