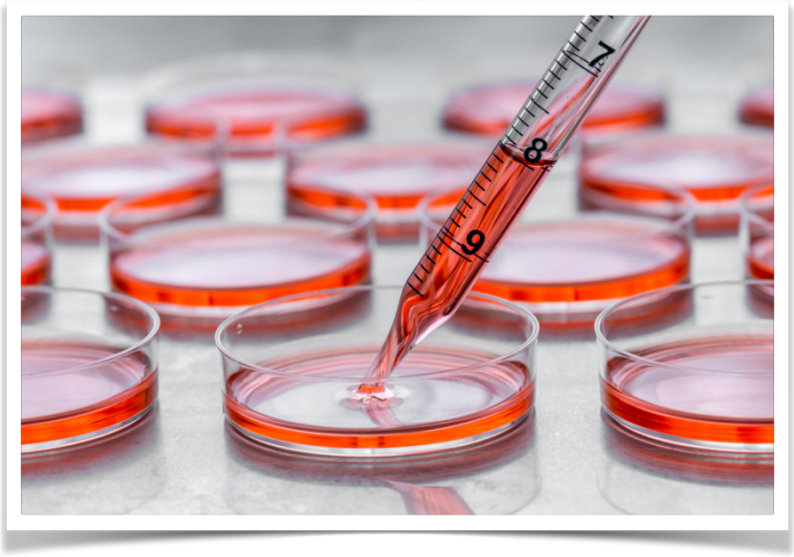
02 – CELLS AS THE BASIS OF LIFE

2.6 Cell Culture



I

1

Cell Culture

2.6.1 Human beings culture cells for a variety of purposes.

☑ Describe techniques of cell culture, and discuss the applications and limitations of contemporary examples.

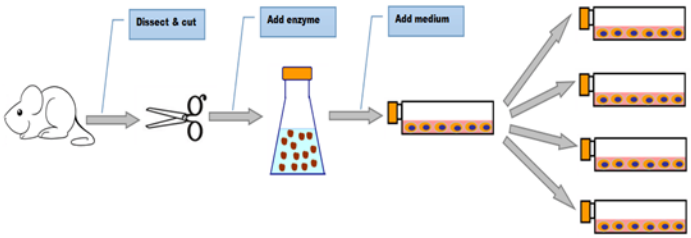


Fig1: the flow chart for primary cell culture

Defining Cell Culturing

- Growing cells outside of their native environment in controlled conditions.

Microorganisms; Animal cells; Plant cells

2

2

Cell Culture

What is a cell line culture?

Cell Line Cultures

- taken from cell cultures of a certain organism desired
- can be indefinitely cultured for many generations
- excellent for ongoing research related to that organism

eg. HeLa Cells - first 'immortal' cell line taken from Henrietta Lacks in 1951.
(these cells behaved differently from other cells; derived from cancerous cells)
https://en.wikipedia.org/wiki/HeLa

Techniques

1. **Dissection**
   - remove cells from source tissue (sterilized)
   - *Physical* - sharp tools to remove cells
   - *Chemical* - use enzymes to digest extracellular matrix & free up cells so they are not stuck together

2. **Conditions for growth**
   - *figure out density of cells in sample and dilute as needed*
   - *Add needed factors for optimum growth; control env.*
   - **sterile!!*

5

Techniques

2. **Conditions for growth**

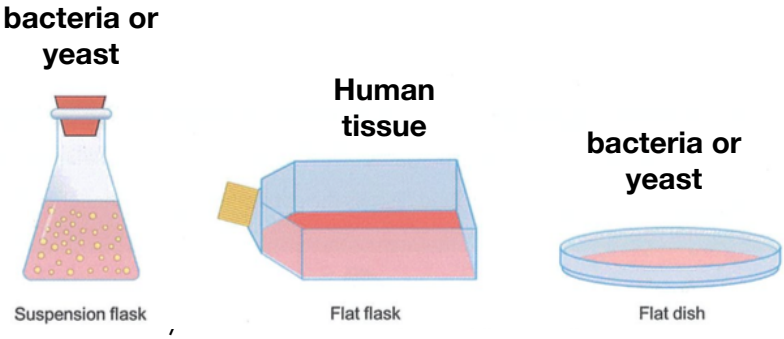
Requirement	Reason(s)
Nutrients	Cells require macronutrients and micronutrients for metabolic reactions involved in growth and development.
Oxygen	Aerobic cells are cultured in the presence of oxygen.
Growth factors	Growth factors and hormones are added in the concentrations that stimulate growth and development.
Osmotic balance	The amount of water and solutes are controlled to ensure an osmotic balance between cells and the extracellular environment.
pH	Cells are cultured in a medium that has the optimum pH for enzyme activity. pH buffers are added to maintain the optimum pH.
Temperature	Cells are cultured in a medium that has the optimum temperature for enzyme activity.
Antibiotics	Antibiotics are added to kill bacteria that would contaminate the culture.

6

Techniques

3. Culture

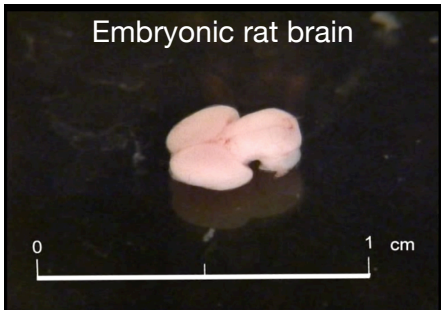
- *incubated in suitable vessel @ optimal temp*
- *Type of vessel - depends on cell type*
  - **anchorage dependent? (eg. skin cells)*
  - **anchorage independent? (eg. blood cells)*



Techniques - Video

This video shows how to prepare rat embryonic cultures of hippocampal ***neurons***, step by step and how the neurons should look like once cultured in vitro.

This video was made by the Fritschy Lab at the University of Zurich.

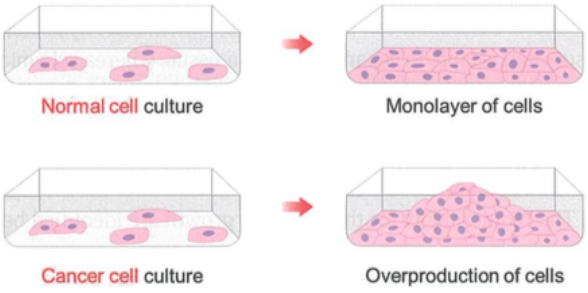


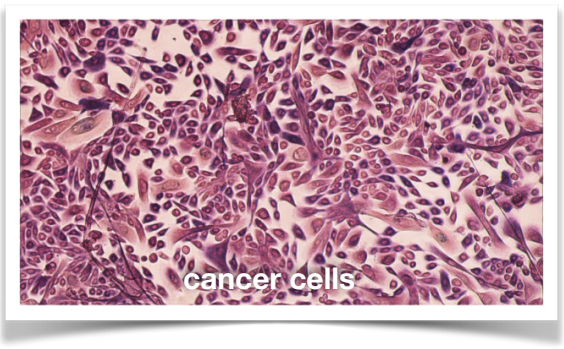
https://www.youtube.com/watch?v=eHDapIC6QvY

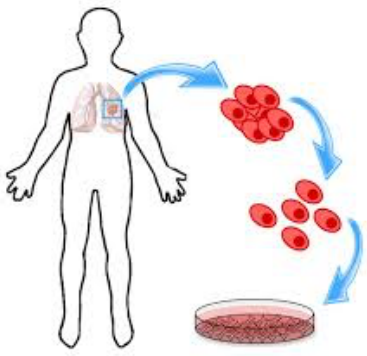
8

Culturing Cancer Cells

Are not restricted by normal dependencies (anchorage, etc)







Limitations of Cell Culture

Advantage	Limitation
The environmental factors can be controlled to ensure optimal growth rate of cells.	Contamination by microbes and other cell types is possible in a laboratory setting.
Is used to effectively study interactions between medicines and different cell types.	Studying the effects of drugs and pathogens on a targeted cell type does not identify all effects of the drug or pathogen in the body of an organism.
Is used by medical scientists to effectively diagnose a disease from a tissue sample obtained from a patient.	Rapid growth rate of cells introduces mutations and genetic variation which is undesirable in a tissue culture.
New antibodies and vaccines can be synthesised and their effects on cells can be studied outside the body.	Studies have revealed differences in protein expression and other metabolic reactions in cultured cell types.
Cultured cells can be preserved and studied for years following the initial growth phase.	Non-cancerous cell lines have a finite capacity for growth by cell division. The morphology and biochemistry of cells changes with age.

Applications

Application	Description
Cytology research	Scientists culture different cell types to study cell structure and function.
Biochemistry research	Scientists culture cells containing specific mutations to study the effect of gene mutation on metabolic pathways.
Genetics research	Scientists have cultured prokaryotes and eukaryotes to study the structure and function of their chromosomes.
Toxicology research	Scientists study the effect of different concentrations of new and existing drugs on the structure and function of cells.
Cancer research	Scientists study the properties of cancer cells as well as the effects of ionising radiation and different drugs to improve targeted radiotherapy and chemotherapy techniques.
Virology research	Scientists study the interactions between viruses and cultured cells for the development of new drugs and vaccines.
Cosmetics research	Scientists study the effects of new cosmetics on cultured cell types to ensure they are safe for use by consumers.
Consumer products	Scientists culture plant cells and tissues to produce drugs, cosmetics, and food products for manufacturing industries.
Food production	Scientists culture bacteria and yeast for the manufacture of cheese, bread, wine, and beer.

Applications

Cell Culture for Crustacean Industry

A breakthrough by a **James Cook University** researcher has closed the gap on the development of a disease-fighting tool in **crustaceans** that has eluded scientists for decades.

The **cell line**, a permanent cell system contained in a bottle, could help wipe out deadly viruses infecting crustaceans and save the world's aquaculture industry from millions of dollars in losses.

In 1987-88 the prawn aquaculture industry in Taiwan was hit by a virus reducing the country's 115,000 tonne industry to 44,000 tonnes.

The development of a cell line will help scientists better understand viruses affecting crustaceans and provide a more sensitive and reliable diagnostic tool, which will not only standardize the system for disease analysis, but also minimize animal experimentation.

Corneal Cell Cultures

Scientists in Australia have developed a technique for growing corneal cells on a thin layer of film in the lab, which can then be implanted into the eye to restore vision lost to corneal damage.

The technique, which has been so far tested on sheep, involves taking a sample of the subject's corneal cells, cultivating them on the synthetic film, and then returning them in greater numbers to the eye – where the regenerated cells restore moistness-pumping functions that keep the cornea healthy and clear.

https://www.sciencealert.com/a-new-invisible-film-made-from-healthy-corneal-cells-could-restore-vision-to-millions

Reproductive Cloning & Micropropagation

Reproductive Cloning:

- used to clone plants and animals
- clone = genetically identical to 'parent'
- clone crops/livestock with desirable qualities
- help food shortage issues
- restore endangered populations

Micropropagation:

- plants often do vegetative propagation when they clone themselves from part of themselves (stem, root, etc)
- Micropropagation does this artificially
- see example right

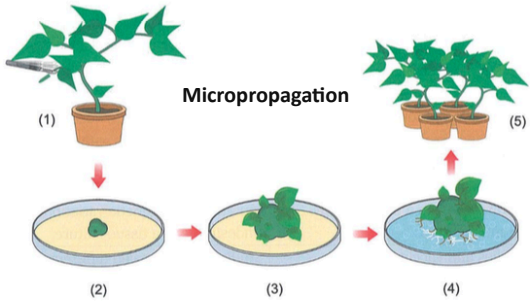


Figure 2.73: Steps in the production of clones using micropropagation.

Step	Description
(1)	A small piece of plant tissue (called an **explant**) is removed from the plant using dissection.
(2)	The explant is transferred to a growth medium containing growth factors that induce cell division.
(3)	Cells in the tissue start to divide forming a shapeless, undifferentiated mass called a **callus**.
(4)	The callus is treated with growth hormones at the appropriate concentrations to induce growth of roots and shoots.
(5)	Established plants are transferred to soil and grown under optimal conditions. Plants cultured in this way are clones.

Micropropagation

Advantages:

Disadvantages:

- reduction of biodiversity (genetic diversity and species diversity)
- increased susceptibility to infection from pathogens

Reproductive Cloning in Animals

Refer to p. 222-223 in SASTA text (and diagrams)
[2020]

15