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" أساسيات الزراعة النسيجية "

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Lecture (1)

The Tissue

A tissue is an ensemble of similar cells from the same origin that together carry out a specific function. Multiple tissue types compose organs and body structures.

Tissue Culture

Cell culture is the complex process by which cells are grown under controlled conditions, generally outside of their natural environment. In practice, the term "cell culture" now refers to the culturing of cells derived from multi-cellular eukaryotes , especially animal cells . However, there are also cultures of plants, fungi, insects and microbes, including viruses, bacteria and protists.

The source of the culture

• Cell lines derived from embryo – contain more stem cells and precursor cells – Greater self renewal than culture from adult

• The identity of cells is defined by its lineage in vivo and by its position in that lineage.

Classification of Cell Cultures

• **Primary Culture**

– Cells taken directly from a tissue to a dish

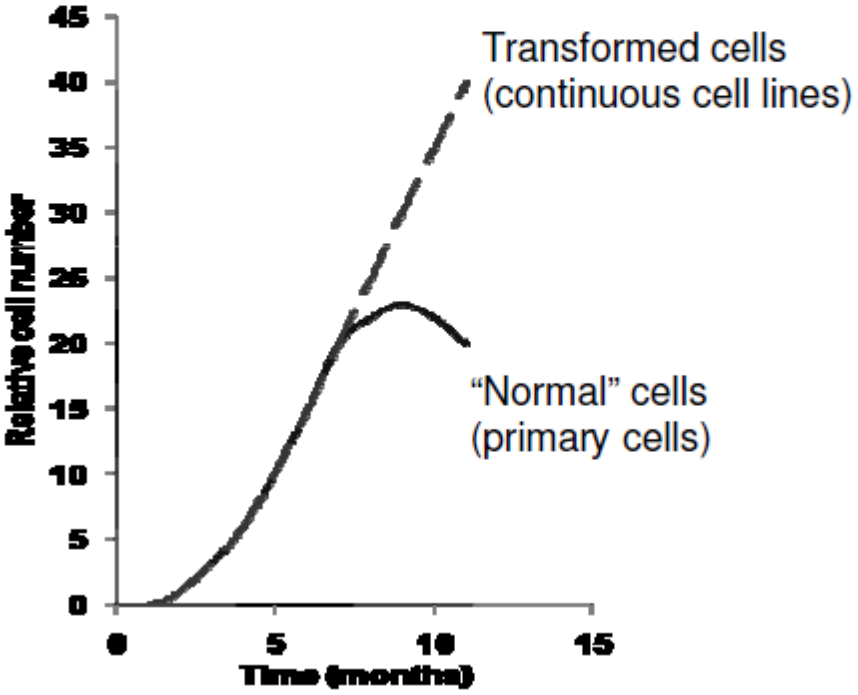
– Can be passages with a limited number of times. After the limit, the cell will die.

• **Culture of establish cell lines**

– Established or immortal cell lines

– Cells taken from a primary culture and passed or divided in vitro. Can grow indefinitely in culture

Growth of normal and transformed cells in culture



Primary cells vs cell lines

Primary Cells	**Cell lines**
-Freshly isolated cells (from tissues)	**- Originally primary, but then transformed so keep growing**
-Hard to culture	**-Easy to culture**
-Heterogeneous (mixed) cell Population	**-Homogeneous (cloned?) cell population**
-Finite number of passages (gradual loss of function)	**-Infinite number of passages (cancer-like)**
-More "physiological"	**-Less "physiological**

Cell line

A cell line is the culture that is produced from subculture of the primary and containing the same genetic makeup that separated from their original tissue .

Monolayer of Cells

When a tissue sample is disaggregated ,either mechanically or enzymitically ,the suspension of cells and small aggregates that is generated will contain a proportion of cells capable of attachment to a solid substrate , forming a ***monolayer*** **(**cell-cell adhesion is regulated by intracellular signalling pathways **)** .

Those cells within the monolayer that are capable of proliferation will then be selected at the first subculture and may give rise to **a *cell line* .**

Isolation of cells

Cells can be isolated from tissues for ex vivo culture in several ways. Cells can be easily purified from blood; however, only the white cells are capable of growth in culture.

Mononuclear cells can be released from soft tissues by enzymatic digestion with enzymes such as **collagenase**, **trypsin**, or **pronase**, which break down the extracellular matrix. Alternatively, pieces of tissue can be placed in growth media, and the cells that grow out are available for culture.

Cells that are cultured directly from a subject are known as **primary cells**. With the exception of some derived from tumors, most primary cell cultures have limited lifespan.

An established or immortalized cell line has acquired the ability to proliferate indefinitely either through random mutation or deliberate modification .

Numerous cell lines are well established as representative of particular cell types.

Maintaining cells in culture

- Cells are grown and maintained at a appropriate **temperature** and gas mixture (typically, 37 °C, 5% CO_2 for mammalian cells) in a cell incubator.
- **Culture conditions** vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes.
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- Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the **cell growth medium**. Recipes for growth media can vary in ***pH***, ***glucose concentration***, ***growth factors*** , and the presence of other ***nutrient components*** .

- Pink color of the medium indicates the right **pH** for the cultured cells.

- The **growth factors** used to supplement media are often derived from the **serum** of animal blood , such as ***fetal bovine serum (FBS)***, ***bovine calf serum***.

Foetal Calf/Bovine Serum (FCS & FBS)

- Growth factors and hormones
- Aids cell attachment
- Binds and neutralise toxins
- Long history of use

❖ **Heat Inactivation (56°C for 30 mins) – why?**

- Destruction of complement and immunoglobulins
- Destruction of some viruses (also gamma irradiated serum).

Suspension - Adhesion

Cells can be grown either in suspension or adherent cultures. Some cells naturally live in suspension, without being attached to a surface, such as cells that exist in the blood stream. There are also cell lines that have been modified to be able to survive in suspension cultures so they can be grown to a higher density than adherent conditions would allow.

Adherent cells require a surface, such as tissue culture plastic , which may be coated with extracellular matrix (such as collagen and laminin) components to increase adhesion properties and provide other signals needed for growth and differentiation. Most cells derived from solid tissues are adherent ; Cells that attach to surfaces in vivo require a surface to attach to in vitro.

• Transformed cells may not require attachment.

Other technical issues

As cells generally continue to divide in culture, they generally grow to fill the available area or volume.

This can generate several issues:

- Nutrient depletion in the growth media
- Changes in pH of the growth media (Pink color of the medium indicates the right pH for the cultured cells.)
- Accumulation of apoptotic/necrotic **(dead) cells**
- Cell-to-cell contact can stimulate **cell cycle arrest**, causing cells to stop dividing, known as **contact inhibition**.
- Cell-to-cell contact can stimulate **cellular differentiation**.

Manipulation of cultured cells

Among the common manipulations carried out on culture cells are **media changes** , **passaging cells** , ect.. These are generally performed using tissue culture methods that rely on aseptic technique.

Aseptic technique aims to avoid contamination with bacteria, yeast , or other cell lines .

Manipulations are typically carried out in a biosafety hood or laminar flow cabinet to exclude contaminating micro-organisms. Antibiotics (e.g. **penicillin and streptomycin**) and antifungals (e.g. nystatin .) can also be added to the growth media.

As cells undergo metabolic processes, acid is produced and the pH decreases. Often, a pH indicator is added to the medium to measure nutrient depletion (Pink color of the medium indicates the right pH for the cultured cells).

Media changes

In the case of adherent cultures, the media can be removed directly by aspiration, and then is replaced. Media changes in non-adherent cultures involve centrifuging the culture and re-suspending the cells in fresh media.

Passaging cells

Most animal cell lines and primary cultures grow as a single thickness cell layer or sheet attachment to a plastic or glass subculture. Once the available subculture surface is covered by cells (a confluent culture) growth slows and then stopped .Thus ,in order to keep the cell healthy

and actively growing ,it is necessary to subculture them at regular intervals .

• Passaging or splitting cells involves transferring a small number of cells into a new vessel.

• Cells can be cultured for a longer time if they are split regularly, as it avoids the senescence associated with prolonged high cell density.

• **Suspension culture**s are easily passaged with a small amount of culture containing a few cells diluted in a larger volume of fresh media.

• For **adherent cultures**, cells first need to be detached involving breaking the bonds or cellular "glue " that attaches the cell to the subculture and to each other ; this is commonly done by using proteolytic enzymes such as trypsin , dispase , or collagenase. Occasionally , these enzyme or dissociation agents are combined with divalent cation chelators such as EDTA (binds calcium and magnesium ions) . The loosened cells are then removed from the culture vessel ,counted ,diluted and subdivided into new vessels.

Cells then reattached ,begin to grow and divide , and , after a suitable incubation period (depending on the initial inoculums size ,growth , conditions and cell line) ,again reach saturation or confluency .At this point , the subcultivation cycle can be repeated .

Passage number

• The number of times the cells have been removed (or “split”) from the

plate and re-plated.

• Always write this on your plate or flask as : **P #**

Tissue culture media

Tissue culture media are necessary to maintain **pH** and **osmolality**, which are essential for **viability**, and to provide the **nutrients** and **energy** required for cell growth.

Cell culture is one of major techniques in the life sciences. It is the general term used for the removal of cells, tissues or organs from an animal or plant and their subsequent placement into an **artificial environment** advantageous to their survival and/or proliferation.

Basic environmental requirements for cells to grow optimally are:

1-controlled temperature .

2-substrate for cell attachment .

3-**appropriate growth medium**

4-**incubator that maintains correct pH and osmolality**.

The most important step in cell culture is selecting appropriate **growth medium** for the in vitro cultivation.

Cell culture media generally comprise an appropriate source of **energy** and **compounds** which regulate the cell cycle.

A typical culture medium is composed of a complement of **amino acids**

Vitamins

inorganic salts

glucose

serum as a source of growth factors

hormones

attachment factors and other nutrients ;

available either as a **powder** or as a **liquid** form from commercial suppliers .

The requirements for these components vary among cell lines, and these differences are partly responsible for the extensive number of medium formulations .

Artificial media

- **Basal media (Primary culture)**

MEM - Minimum Essential Medium
DMEM - Dulbecco's Modified Eagle's Medium

- **Complex media** (Supports wide range of mammalian cells)

RPMI-1640- Roswell Park Memorial Institute medium

IMDM - Iscove’s Modified Dulbecco’s Medium

Basic Components of Culture Media

Each component performs a specific function, as described below:

Buffering systems

Regulating pH is critical for optimum culture conditions and is generally achieved by adding **sodium carbonate** and **HEPES** that maintained pH within **7.2 ± 0.2** to neutralize **CO2** resulted from the respiration and metabolism of the cultured cells .

Phenol red (pH indicator)

Most of the commercially available culture media include phenol red as a **pH indicator**, which allows constant monitoring of pH . During the cell growth, the medium changes color as pH is changed due to the metabolites released by the cells.

At low pH levels (**6.5**), phenol red turns the medium yellow ,

pH **7.0** turns the medium orange ,

Medium is **bright red** for "pH **7.4**"

while at higher pH levels (**7.8**) it turns the medium purple. , the

Inorganic salt

Inorganic salt (HCO3, Mg , ,K, Na, Cl, SO4 ,PO4) in the media help to retain the **osmotic balance** and help in regulating **membrane potential** by providing Sodium Na, Potassium K, and Calcium ions Ca .

Amino Acids

Amino acids are the building blocks of proteins, and thus are obligatory ingredients of all known cell culture media. **Essential amino acids** must be included in the culture media as cells can not synthesize these by themselves.

They are required for **the proliferation of cells** and their concentration determines the **maximum achievable cell density**.

L-glutamine, an essential amino acid, is particularly important . **L-glutamine** provides **Nitrogen** for NAD, NADPH and nucleotides and serves as a **secondary energy source** for metabolism.

Carbohydrates

Carbohydrates in the form of **sugars** are the major **source of energy**.

Most of the media contain **glucose** and **galactose**, however, some contain **maltose** and **fructose**.

Proteins and Peptides

The most commonly used proteins and peptides are **albumin**, **transferrin**, and **fibronectin**. **Serum** is a rich source of proteins

and includes albumin, aprotinin, fetuin , fibronectin and transferrin.

Fatty Acids and Lipids

They are particularly important in serum-free media as they are generally present in **serum**.

Vitamins

Many vitamins are essential for growth and proliferation of cells. Vitamins cannot be synthesized in sufficient quantities by cells and are therefore important supplements required in tissue culture.

Again **serum** is the major source of vitamins in cell culture, however, media are also enriched with different vitamins making them suitable for a particular cell line. The B group vitamins are most commonly added for growth stimulation.

Trace Elements

Trace elements are often supplemented to serum-free media to replace those normally found in **serum**. Trace elements like **copper**, **zinc**, **selenium** and **tricarboxylic acid** intermediates are chemical elements that are **needed in minute amounts** for proper cell growth . These **micronutrients** are essential for many biological processes, e.g. the maintenance of the functionality of enzymes.

Media Supplements

The complete growth media recommended for certain cell lines requires additional components which are not present in the basal media and serum. These components, supplements, help enhance proliferation and maintain normal cell metabolism .

Although supplements like **hormones**, **growth factors** and **signaling substances** are required for normal growth of some cell lines, it is always best to take the following precautions:

since the addition of supplement can **change the osmolality** of the complete growth media which can negatively affect the growth of cells, it is always best to **recheck the osmolality** after supplements are added.

Antibiotics

Although not required for cell growth, antibiotics are often used to control the growth of bacterial and fungal contaminants .

Serum in Media

Serum is a complex mix of albumins, growth factors and growth inhibitors .

Serum is one of the most important components of cell culture media and serves as a source for

amino acids

- Proteins like fibronectin, which promote attachment of cells to the substrate.
- several **binding proteins** like albumin, transferrin, which can carry other molecules into the cell. For example: albumin carries lipids, vitamins, hormones, etc into cells.

vitamins (particularly fat-soluble vitamins such as A, D, E, and K)

carbohydrates

lipids

- hormones and growth factors involved in growth promotion and specialized cell function.

- minerals, like Na+, K+, Zn2+, Fe2+, etc.

trace elements.

--It provides **protease inhibitors** which protect cells from preolysis.

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--It increases the viscosity of medium and thus, protects cells from mechanical damages during agitation of suspension cultures.

--It also acts a buffer.

RPMI-1640 Medium

Preparation of RPMI-1640 culture medium is done under aseptic condition using laminar air flow hood, 10.4 gm / L RPMI-1640 powder with Hepes, with L- glutamine , without sodium bicarbonate is dissolved in 900 ml deionised distilled water (ddH2O). Then 100 I.U/ml benzathin penicillin and 100 µg/ml streptomycin, 2 g / L Sodium bicarbonate and pH adjusted to 7.2. 10% Fetal calf serum is added.

The volume is brought up to 1000 ml using sterile ddH2O and then the medium is sterilized by nalgen filter (0.22µm) and distributed into sterile vial and stored at -20°C until use. One to two tubes are incubated at 37°C for 72 hr. as check sterility.

Phytohaemagglutinin (PHA)

This solution is prepared by dissolving 5mg of PHA-M in 10 ml sterile ddH2O to get a final concentration of 0.5 mg/ml and stored at -20°C until use.

The blood cells growth is stimulated with 0.2 mg/ml PHA-M immediately after culturing.