

What is Cell & Tissue Culture

- Tissue culture is the general name for the removal of cells, tissues or organs from an animal or plant and their subsequent placement into artificial environment conducive to growth
- This environment usually consists of a suitable glass or plastic culture vessel containing a liquid or semi-solid support medium that supplies the nutrients essential for survival and growth
- When the cells are removed from the organ fragments, thus disrupting their normal relationship with neighboring cells, it is called cell culture

Classes of Culture Cells

- Cultures of animal cells are usually divided into 3 classes:
 1. Primary cells
 2. Cell strains
 3. and cell lines

1- Primary Culture

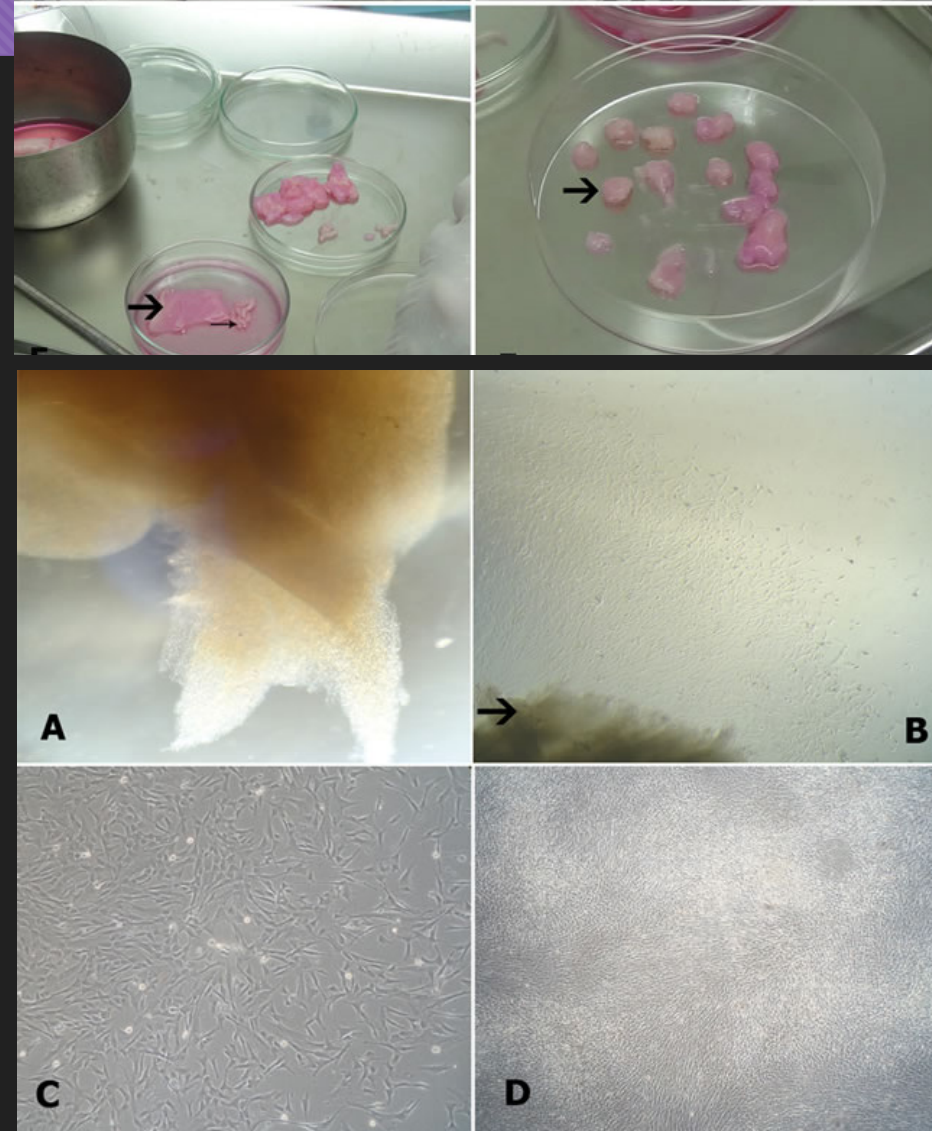
- When cells are surgically removed from an organism and placed into a suitable culture environment they will attach, divide and grow
- Most of the primary culture cells have a finite lifespan of 5-10 divisions in vitro
- Due to their limited lifespan, one cannot do long-term experiments with these cells
- Primary cells are considered by many researchers to be more physiologically similar to in vivo cells

1- Primary Culture

- There are two basic methods for obtaining primary culture:

1. Explant cultures:

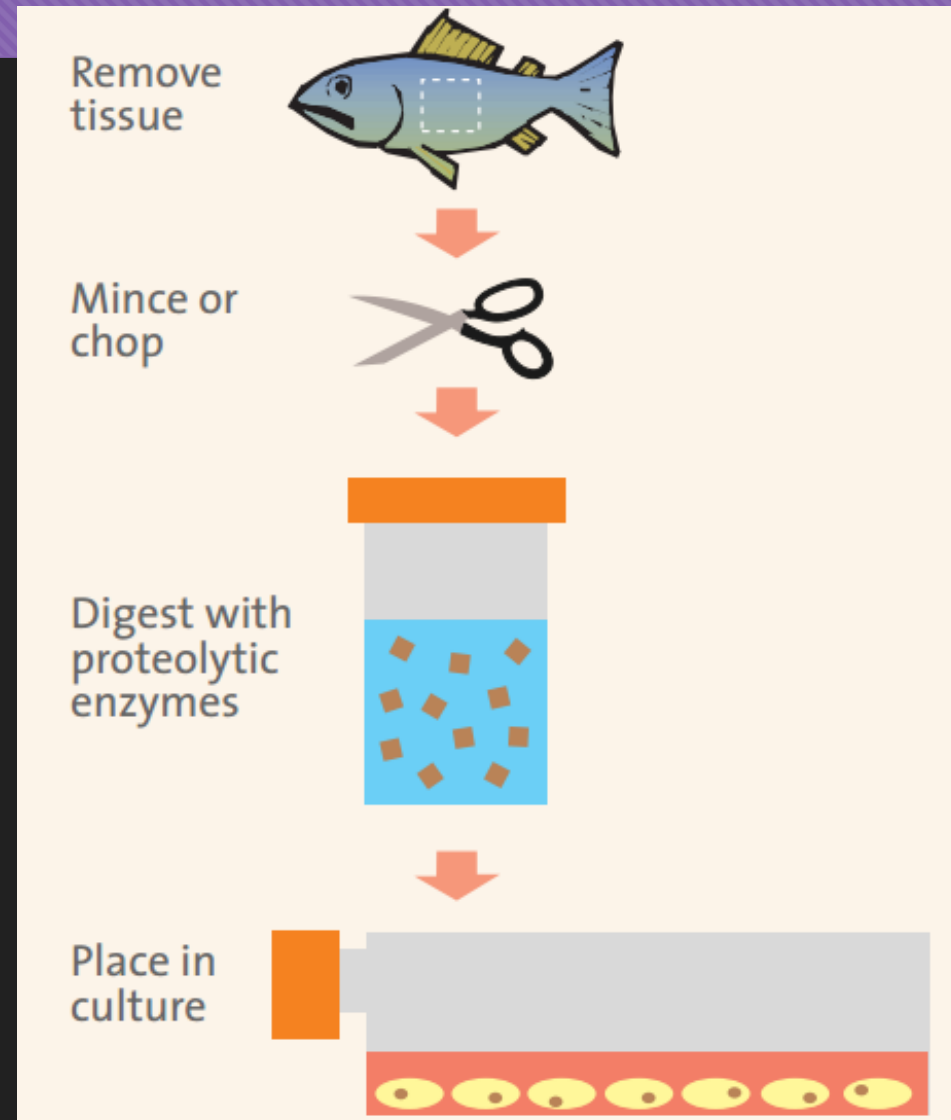
- Small pieces of tissue are attached (using plasma clots or fibrinogen) to a glass or treated plastic culture vessel and immersed in culture medium
- After a few days individual cells will move from the tissue explant out onto the culture vessel surface or substrate where they will begin to divide and grow



1- Primary Culture

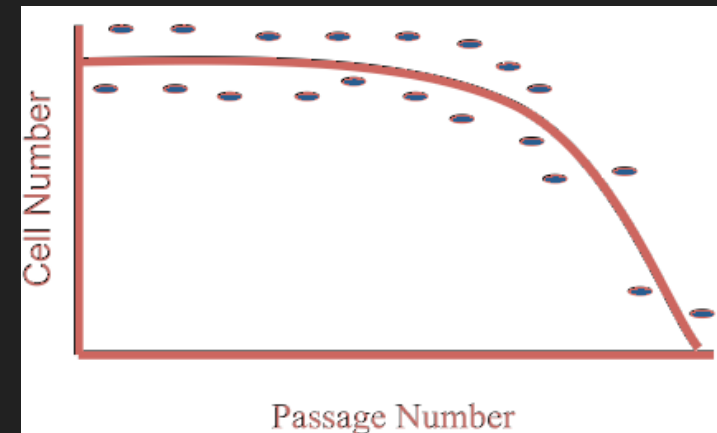
2. Enzymatic dissociation:

- More widely used
- speeds up the process by adding digesting (proteolytic) enzymes such as trypsin or collagenase to the tissue fragments to dissolve the cement holding the cells together
- This creates a suspension of single cells that are then placed into culture vessels containing culture medium and allowed to grow and divide



Hayflick's Phenomenon

- Cells will continue to grow and divide normally for a limited number of passages
- When they get to a certain point even if they are given the appropriate nutrients, they simply stop dividing and will eventually die
- There appears to be a correlation between the maximal number of passages and aging
- The number of passages decreases when cells are harvested from older individuals



2- Cell Strains

- Cell strains are cells that have been adapted to culture but, unlike cell lines, have a finite division potential
- Upon serial transfers of primary cells, a gradual selection may occur until a particular cell type becomes predominant
- If these cells continue to grow at a constant rate over successive passages, these primary cells are referred to as a cell strain
- These cells have a finite lifespan of 40-60 divisions in vitro
- They are useful in vaccine production

3- Cell Lines

- If the cells in a cell strain undergo a transformation process (spontaneous or induced changes in karyotype, morphology or growth properties) that makes them "immortal" (able to divide indefinitely) they are called a cell line
- It is not known how a diploid cell strain becomes a cell line, although this event may be mimicked by infection with oncogenic viruses or by exposure to chemical carcinogens
- Cell Lines often have abnormal chromosome numbers and maybe tumorigenic when inoculated into susceptible animals
- Cell lines that have been derived from tumors often do not exhibit contact-inhibition (inhibition of growth under crowded conditions), but rather continue to pile-up

Transformation of Cells

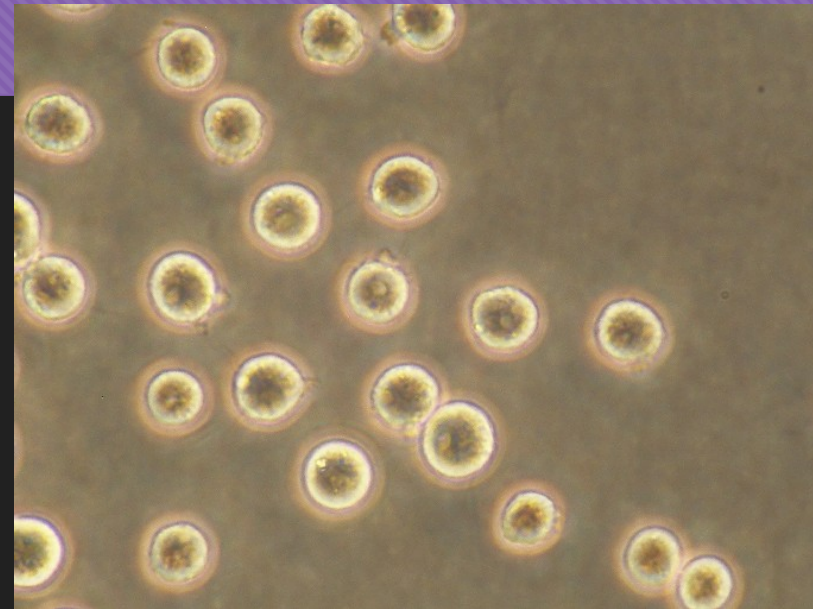
- Transformed, Infinite or Established Cells
- Changed from normal cells to cells with many of the properties of cancer cells
- Some of these cell lines have actually been derived from tumors or are transformed spontaneously in culture by mutations
- Chemical or gamma ray treated cells can become infinite with loss of growth factors
- Viral infection with SV40 T antigen can insert oncogenes and lead to gene alteration
- No matter how transformation occurred, the result is a cell with altered functional, morphological, and growth characteristics

Cell Culture Systems

- Cells may be loosely divided into two main types:

1- Suspension cell culture (Anchorage-independent)

- derived from cells which can divide and survive without being attached to a substrate,
- e.g. cells of haemopoietic lineage
- Can be maintained in culture vessels that are not tissue-culture treated,
- requires agitation for adequate gas exchange
- Easier to passage



Cell Culture Systems

2- Adherent cell culture (Anchorage-dependent)

- must adhere to a surface to survive
- Form monolayers
- e.g. cells derived from different tissue (breast, liver)
- Growth is limited by surface area
- Will cease proliferating once they become confluent (completely cover the surface of cell culture vessel)
- Cells are dissociated enzymatically or mechanically from surface



Growth Cycle in Attachment Culture

- Eukaryotic cells in attachment culture have a characteristic growth cycle similar to bacteria
- The growth cycle is typically divided into three phases:

1- Lag Phase

- This is the time following subculture and reseeding during which there is little evidence of an increase in cell number
- It is a period of adaptation during which the cell replaces elements lost during trypsinization, attaches to the surface, and spreads out

Growth Cycle in Attachment Culture

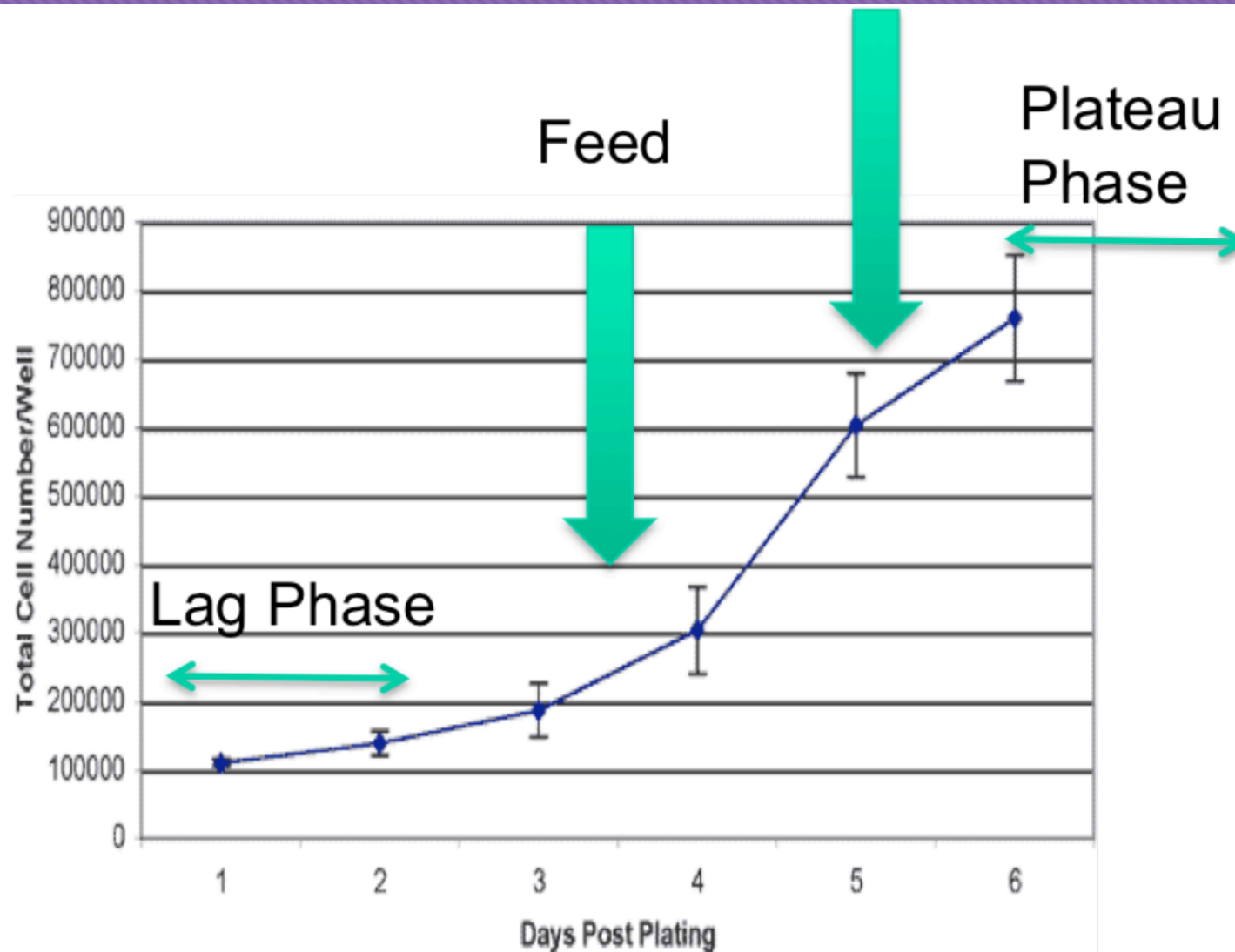
2- Log Phase

- This is the period of exponential increase in cell number
- The length of the log phase depends on the seeding density, the growth rate of the cells
- It is the optimal time for sampling since the population is at its most uniform and viability is high

3- Plateau Phase

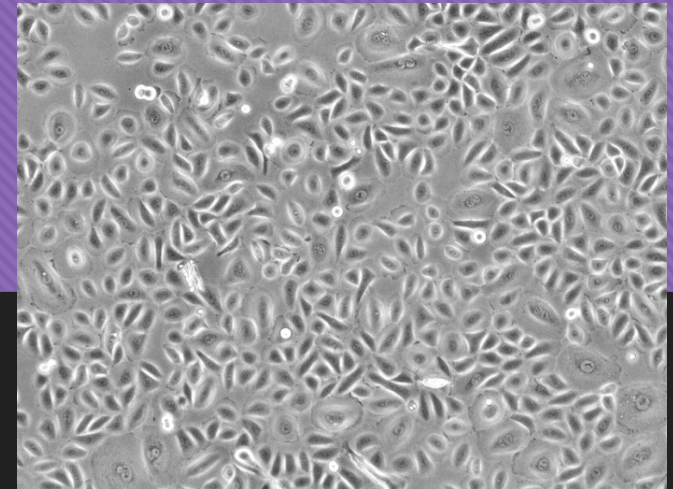
- Toward the end of the log phase, the culture becomes confluent
 - All the available growth surface is occupied and all the cells are in contact with surrounding cells
- Following confluence the growth rate of the culture is reduced, and in some cases, cell proliferation ceases almost completely
- At this stage, the culture enters the plateau (or stationary) phase, and the growth fraction falls

Growth Cycle in Attachment Culture

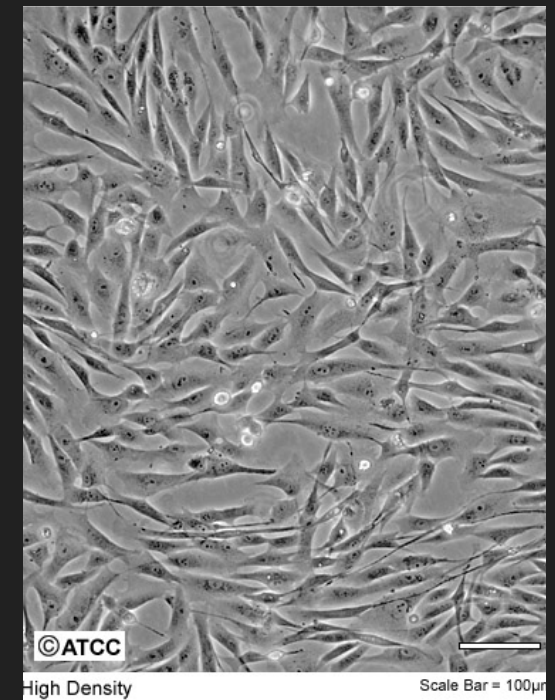


Morphology of Cells

- Cultured cells are usually described based on their morphology (shape & appearance), there are two basic morphologies:
 1. Epithelial-like:
 - cells that appear flattened and polygonal in shape
 2. Fibroblast-like:
 - cells that appear thin and elongated
- Culture conditions play an important role in determining shape and that many cell cultures are capable of exhibiting multiple morphologies



Human Conjunctival Epithelial Cells (HConEpiC) - Phase contrast, 100x.



***Homo sapiens*, human, Foreskin**

Basic Requirements For Successful Cell Culture

1. The first necessity is a well-established and properly equipped cell culture facility. All facilities should be equipped with the following:
 - A certified biological safety cabinet
 - protects both the cells in culture and the worker from biological contaminants
 - A centrifuge, preferably capable of refrigeration
 - A microscope for examination of cell cultures and for counting cells
 - And a humidified incubator set at 37°C with 5% CO₂ in air
 - A 37°C water bath filled with water containing inhibitors of bacterial and fungal growth can also be useful if warming of media prior to use is desired

Basic Requirements For Successful Cell Culture

2. The second requirement for successful cell culture is the practice of sterile technique
 - Prior to beginning any work, the biological safety cabinet should be turned on and allowed to run for at least 15 min to purge the contaminated air
 - All work surfaces within the cabinet should be decontaminated with an appropriate solution;
 - 70% ethanol or isopropanol are routinely used for this purpose
 - Any materials required for the procedure should be similarly decontaminated and placed in or near the cabinet
 - This is especially important if solutions have been warmed in a water bath prior to use
 - The worker should put on appropriate personnel protective equipment for the cell type in question

Basic Requirements For Successful Cell Culture

- Gloved hands should be sprayed with decontaminant prior to putting them into the cabinet and gloves should be changed regularly if something outside the cabinet is touched
- Care should be taken to ensure that anything coming in contact with the cells of interest, or the reagents needed to culture and passage them, is sterile (either autoclaved or filter-sterilized)

Basic Requirements For Successful Cell Culture

3. A third necessity for successful cell culture is appropriate, quality controlled reagents and supplies
 - There are numerous suppliers of tissue culture media and supplements
 - Examples include:
 - Invitrogen (www.invitrogen.com),
 - Sigma–Aldrich (www.sigmaaldrich.com),
 - BioWhittaker (www.cambrex.com),
 - and StemCell Technologies Inc. (www.stemcell.com).
 - Similarly, there are numerous suppliers of the plasticware needed for most cell culture applications (i.e., culture dishes and/or flasks, tubes, disposable pipets)

Basic Requirements For Successful Cell Culture

4. The final necessity for successful cell culture is the knowledge and practice of the fundamental techniques involved in the growth of the cell type of interest
 - The majority of cell culture carried out by investigators involves the use of various non-adherent or adherent continuously growing cell lines
 - These cell lines can be obtained from reputable suppliers such as:
 - the American Tissue Type Collection (ATCC; www.atcc.org)
 - or DSMZ (the German Collection of Microorganisms and Cell Cultures) (www.dsmz.de/mutz/mutzhome.html)
 - Alternatively, they can be obtained from collaborators
 - Regardless of the source of the cells, it is advisable to verify the identity of the cell line and to ensure that it is free of mycoplasma contamination

Cell Culture Medium

- Cells have complex nutritional requirements that must be met to permit their propagation in vitro
- Different types of cells have different growth requirements and a number of chemically-defined formulations have been developed that support the growth of a variety of established cell lines
- Although some serum-free media are available and some cell lines have been adapted to growing in such a medium, most cell lines require the addition of 5-10% serum as a supplement to promote cellular multiplication
- Fetal Bovine Serum (FBS) is often the best to use

Cell Culture Medium

1- The various nutrients required are:

- glucose,
- fats and fatty acids,
- lipids, phospholipids and sulpholipids,
- ATP and amino acids
- Vitamins
- Minerals

2- Serum:

- Serum can provide various growth factors, hormones and other factors needed by the most mammalian cells for their long term growth and metabolism

Cell Culture Medium

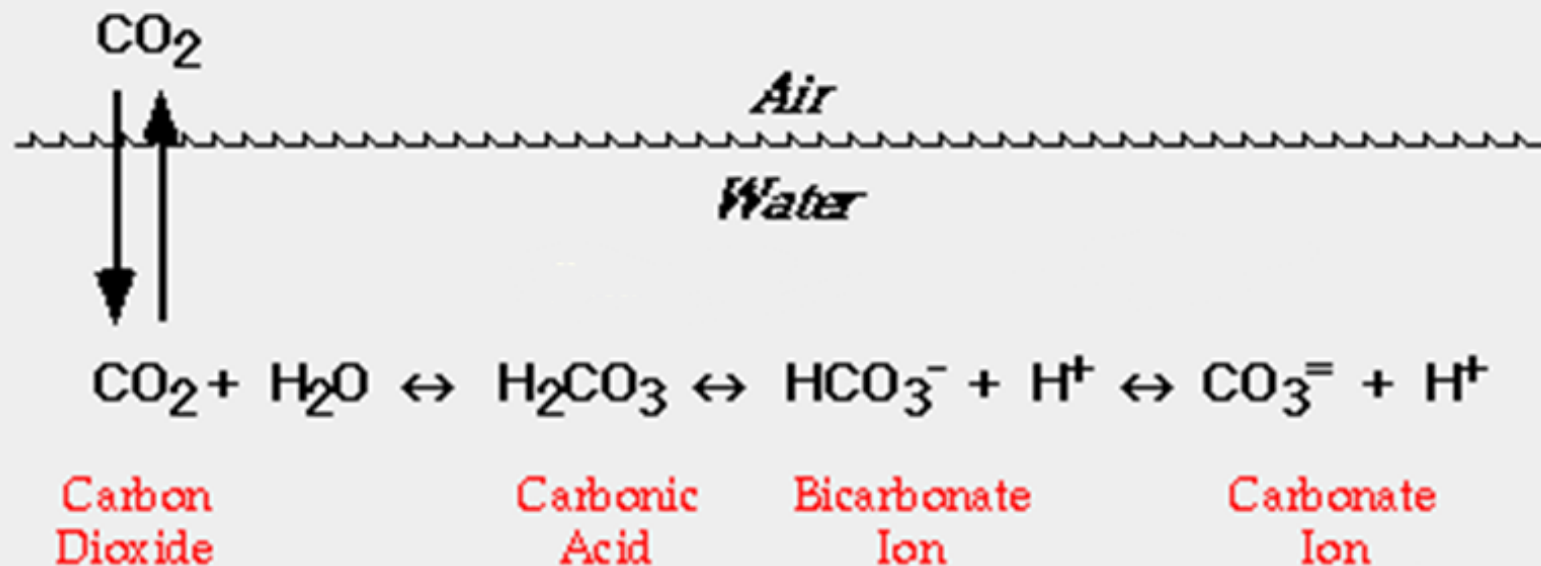
○ L-Glutamine

- L-Glutamine is an essential amino acid required by virtually all mammalian cells grown in culture
- It is used for protein production, as an energy source, and in nucleic acid metabolism
- It is also more labile in liquid cell culture media than other amino acids
- The rate and extent of L-glutamine degradation are related to storage temperatures, age of the product, and pH

Buffering in Cell Culture

- A pH indicator may be Included in the original formulation to permit direct observation of the pH of the medium
- Optimum pH between 7.2 to 7.4 is generally needed for mammalian cells

The Carbonate-Bicarbonate Buffer System



Buffering in Cell Culture

- Generally in the cell culture medium pH indicator, commonly phenol red is used to analyze the pH of environment in which cells are growing
- Phenol red is:
 - yellow in acidic medium (pH 6.8),
 - tomato red at neutral pH (7.0),
 - red at an alkaline pH (7.4)
 - and blue at increased basicity (pH 7.6)
 - and finally purple at high pH



Supplements to Medium: Antibiotics

- Prevention of contamination by the different microorganisms (bacteria, mycoplasma and fungi) is the most important part of all animal cell culture
- The risk of contamination during culture can be avoided by adding different antibiotics, such as:
 - penicillin (100 U/ml) for bacteria,
 - streptomycin (100 mg/ml) for bacteria,
 - or gentamycin (50mg/ ml) for bacteria,
 - and nystatin (50mg/ml) for fungi and yeast
- The routine use of antibiotics is generally not recommended because:
 - it may lead to a relaxation of aseptic technique
 - resistant microorganisms may develop
 - microbial growth may be controlled but biochemical alteration may be produced

Temperature & Humidity

- **Temperature**

- Optimum temperature is also required for the proper growth of the cell
- The optimum temperature of mammal is 37°C

- **Humidity**

- Proper humidity is also essential for cell growth as humidity distribution indirectly also has effect on temperature
- For cell growth 100% humidity is essential to reduce evaporation

Storage of Medium

- Once prepared, the cell culture medium has to be properly stored
- For long-term storage, it should be frozen without NaHCO_3
- On a short-term basis the medium should be kept at 4°C and warmed up to 37°C only for the time necessary to perform a given experiment

Culture Vessels

- Culture vessels provide a contamination barrier to protect the cultures from the external environment while maintaining the proper internal environment
- For anchorage-dependent cells, the vessels provide a suitable and consistent surface for cell attachment
- Other characteristics of vessels include easy access to the cultures and optically clear viewing surfaces

Culture Vessels



○ Flasks

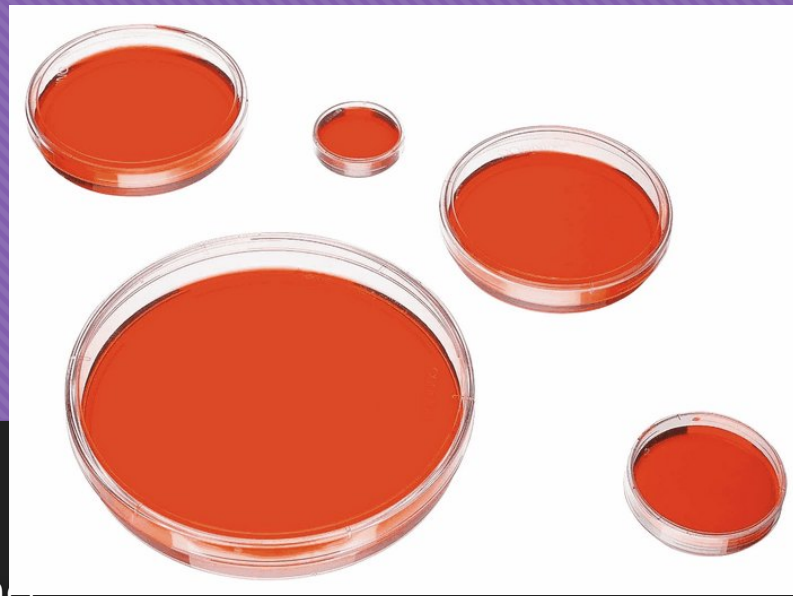
- Plastic flasks are available with a range of growing areas, a variety of shapes, with several different neck designs
- Flasks surfaces are specially treated for growing anchorage-dependent cells



Description	Growth area (cm ²)	Recommended working volume (mL)	Cell yield*
T-25	25	5 – 10	2.5×10^6
T-75	75	15 – 25	7.5×10^6
T-150	150	30 - 50	15×10^6
T-175	175	35 - 60	17.5×10^6
T-225	225	45 - 75	22.5×10^6

*Cell line dependent. Based upon a density of 1×10^5 cells/cm².

Culture Vessels



○ Cell culture dishes

- Cell culture dishes offer the best economy and access to the growth surface
- Cell culture dishes surfaces are specially treated for growing anchorage-dependent cells

Description	Growth area (cm ²)	Recommended working volume (mL)	Cell yield*
35	8	1 - 2	0.8×10^6
60	21	4 - 5	2.1×10^6
100	55	10 - 12	5.5×10^6
150	148	28 - 32	14.8×10^6

*Cell line dependent. Based upon a density of 1×10^5 cells/cm².

Culture Vessels

- **Multiwell plates**

- Multiwell plates offer significant savings in space, media, and reagents when compared to an equal number of dishes



Culture Vessels

- **Surface Coatings**

- Most tissue culture work uses disposable polystyrene vessels
- The vessel surface is treated to render it hydrophilic
- Most cell lines are cultivated on treated plastic surfaces in dishes or flasks
- Some fastidious cell lines require further treatment of the growth surface before they will attach and proliferate
- The most common techniques include coating the surface with serum, collagen, laminin, gelatin, poly-L-lysine, or fibronectin