Cell Culture
part 2

Methods

Growth Requirements

The culture media used for cell cultures are generally quite complex, and culture condition widely varies for each cell type. However, media generally include amino acids, vitamins, salts (maintain osmotic pressure), glucose, a bicarbonate buffer system (maintains a pH between 7.2 and 7.4), growth factors, hormones, $O_2$and CO_2. To obtain best growth, addition of a small amount of blood serum is usually necessary, and several antibiotics, like penicillin and streptomycin are added to prevent bacterial contamination.

Temperature varies on the type of host cell. Most mammalian cells are maintained at 37°C for optimal growth, while cells derived from cold-blooded animals tolerate a wider temperature range (i.e. 15°C to 26°C). Actively growing cells of log phage should be used which divide rapidly during culture.

note : Fetal bovine serum add to obtain best growth

Components of cell culture media[edit]

Component	Function
Carbon source (glucose/glutamine)	Source of energy
Amino acid	Building blocks of protein
Vitamins	Promote cell survival and growth
Balanced salt solution	An isotonic mixture of ions to maintain optimum osmotic pressure within the cells and provide essential metal ions to act as cofactors for enzymatic reactions, cell adhesion etc.
Phenol red dye	pH indicator. The color of phenol red changes from orange/red at pH 7-7.4 to yellow at acidic (lower) pH and purple at basic (higher) pH.
Bicarbonate /HEPES buffer	It is used to maintain a balanced pH in the media

HEPES buffer: 4-(2-Hydroxyethyl)Piperazine-1-Ethanesulfonic Acid

Advantage and disadvantages of using serum with culture media

Advantages of serum in media	Disadvantages of serum in media
Serum contains various growth factors and hormones which stimulates cell growth and functions.	Lack of uniformity in the composition of serum
Helps in the attachment of cells	Testing needs to be done to maintain the quality of each batch before using
Acts as a spreading factor	May contain some of the growth inhibiting factors
Acts as a buffering agent which helps in maintaining the pH of the culture media	Increase the risk of contamination
Functions as a binding protein	Presence of serum in media may interfere with the purification and isolation of cell culture products
Minimizes mechanical damages or damages caused by viscosity	

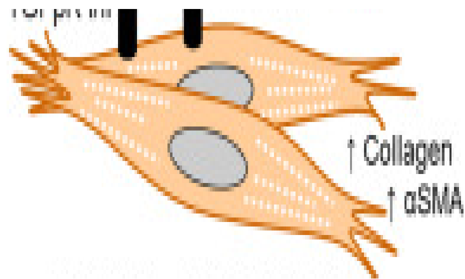
Process to obtain primary cell culture

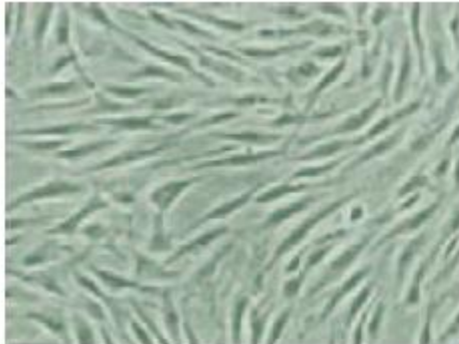
Primary cell cultures are prepared from fresh tissues. Pieces of tissues from the organ are removed aseptically; which are usually minced with a sharp sterile razor and dissociated by proteolytic enzymes (such as trypsin) that break apart the intercellular cement. The obtained cell suspension is then washed with a physiological buffer (to remove the proteolytic enzymes used). The cell suspension is spread out on the bottom of a flat surface, such as a bottle or a Petri dish. This thin layer of cells adhering to the glass or plastic dish is overlaid with a suitable culture medium and is incubated at a suitable temperature.

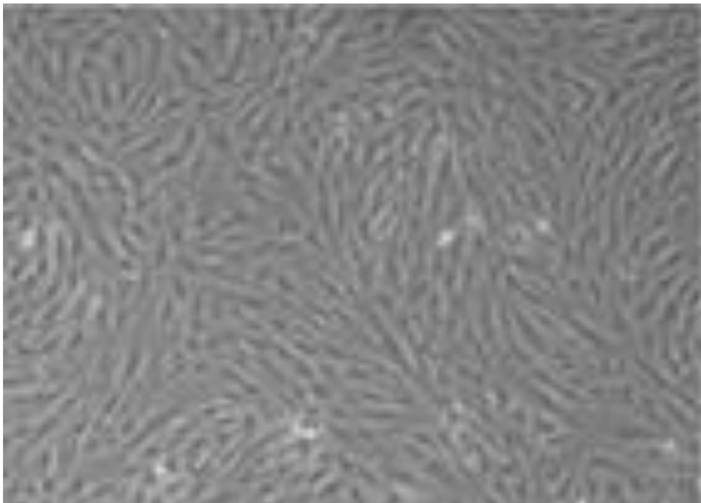
Cell culture morphology

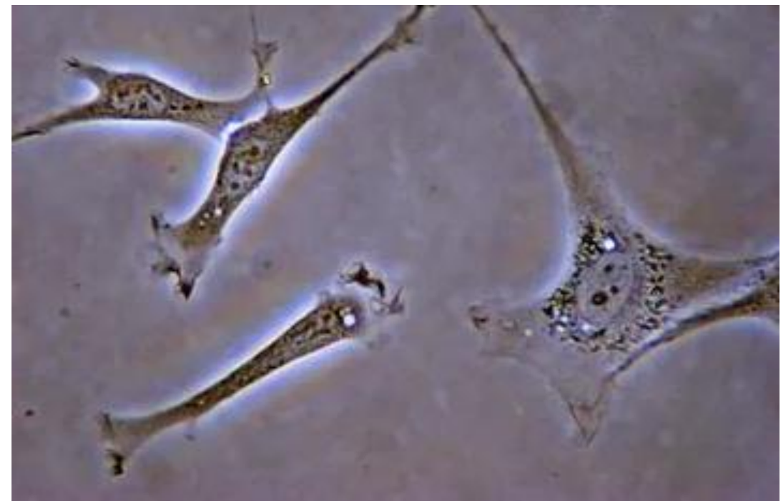
Cells in culture can be divided in to three basic categories based on their shape and appearance (i.e., **morphology**).

- **Fibroblastic** (or fibroblast-like) cells are bipolar or multipolar, have elongated shapes, and grow attached to a substrate.

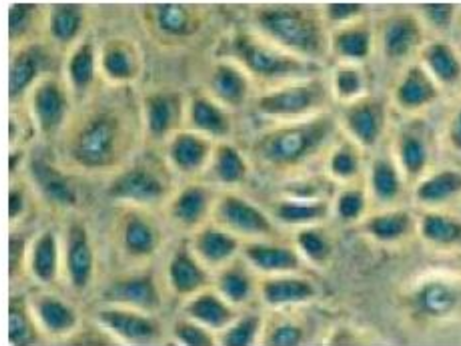


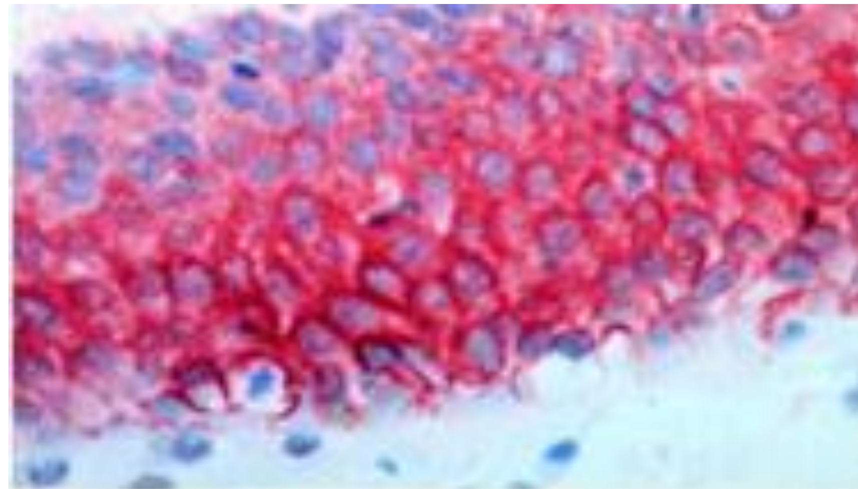


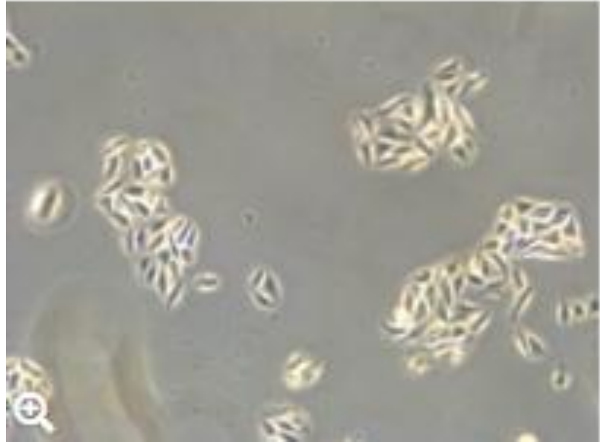
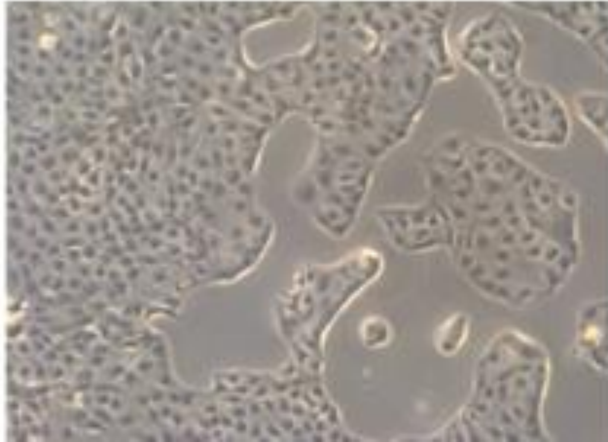
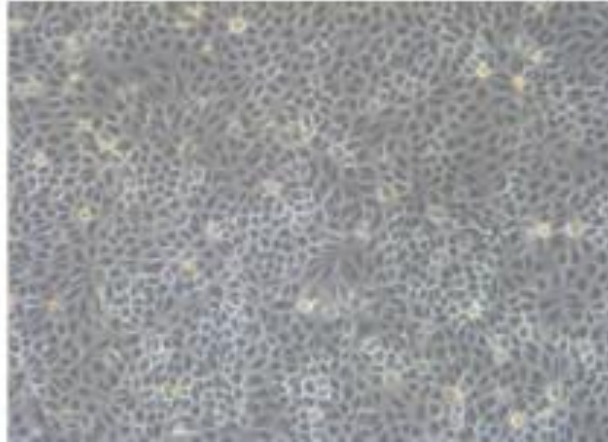




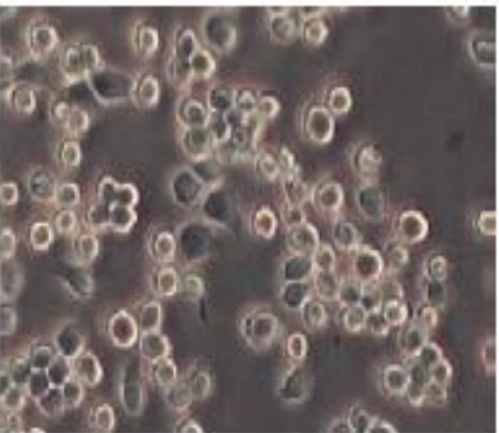
- **Epithelial-like** cells are polygonal in shape with more regular dimensions, and grow attached to a substrate in discrete patches.

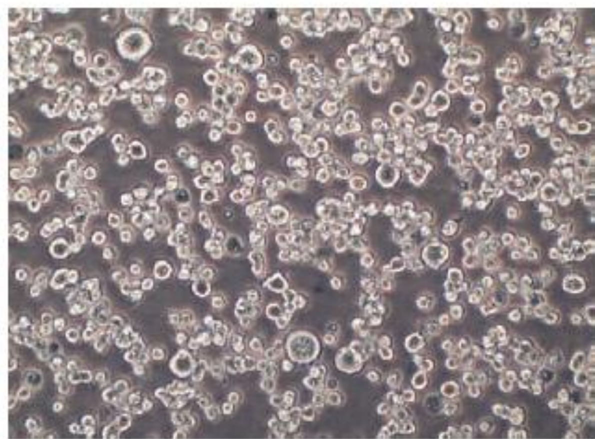




- **Lymphoblast-like** cells are spherical in shape and usually grown in suspension without attaching to a surface.





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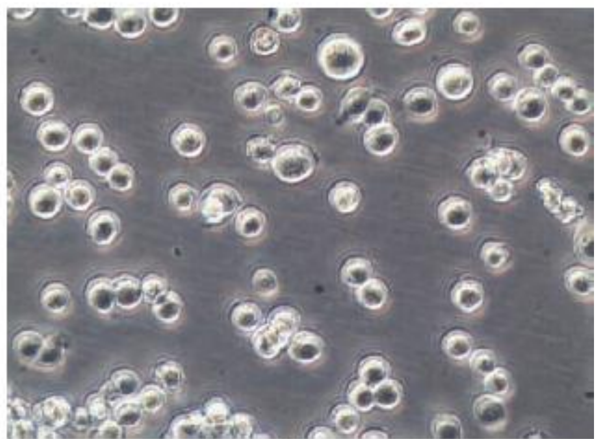


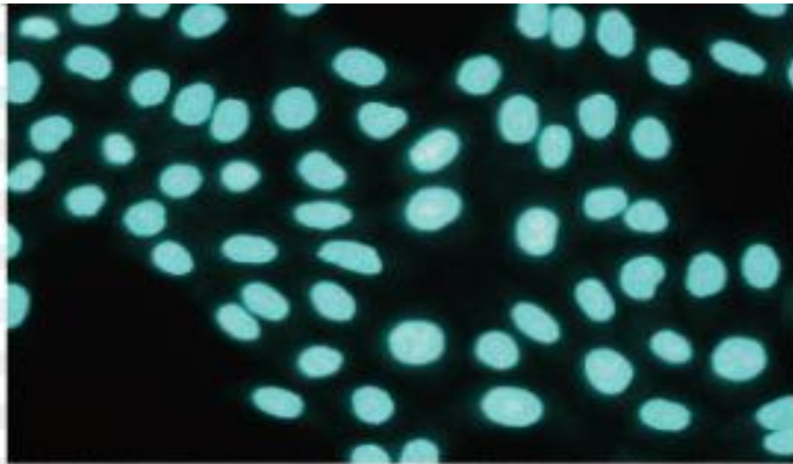
B

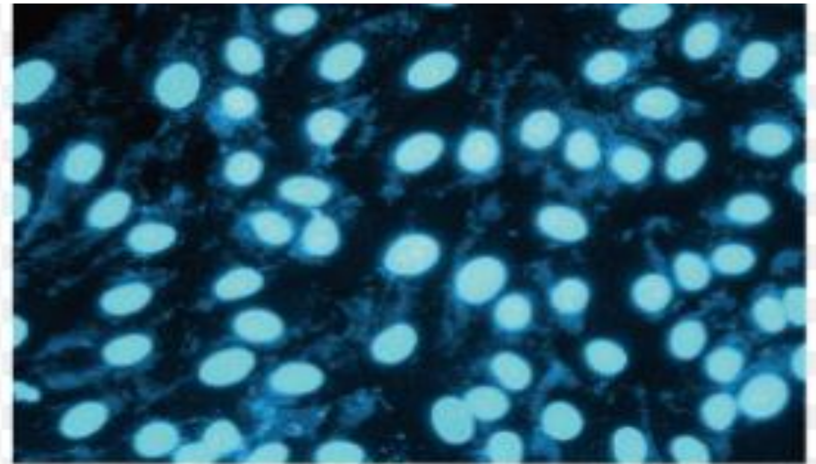
Aseptic techniques

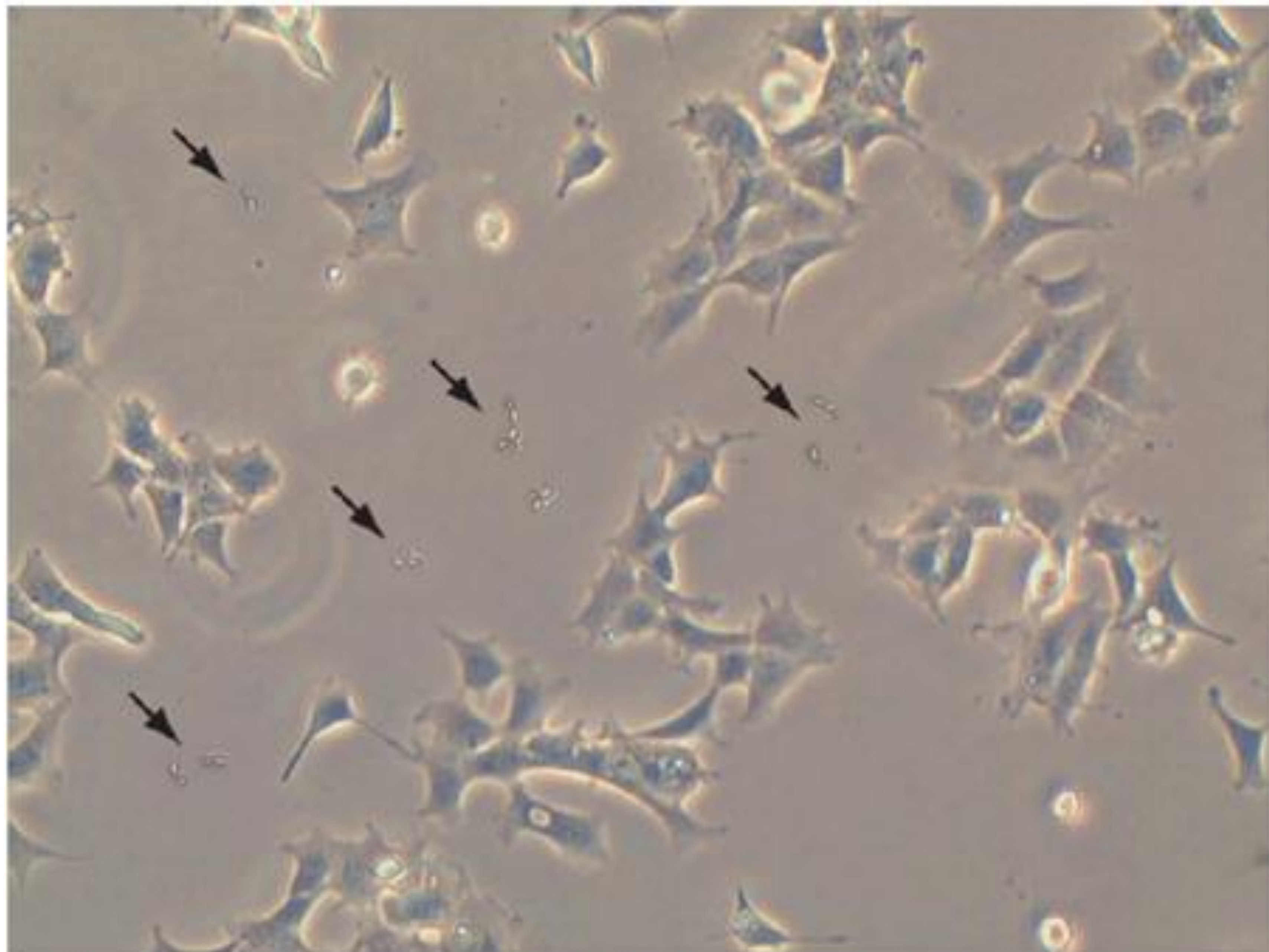
Bacterial infections, like Mycoplasma and fungal infections commonly occur in cell culture creating a problem to identify and eliminate. Thus, all cell culture work is done in a sterile environment with proper aseptic techniques. Work should be done in laminar flow with constant unidirectional flow of HEPA filtered air over the work area. All the material, solutions and the whole atmosphere should be of contamination-free.

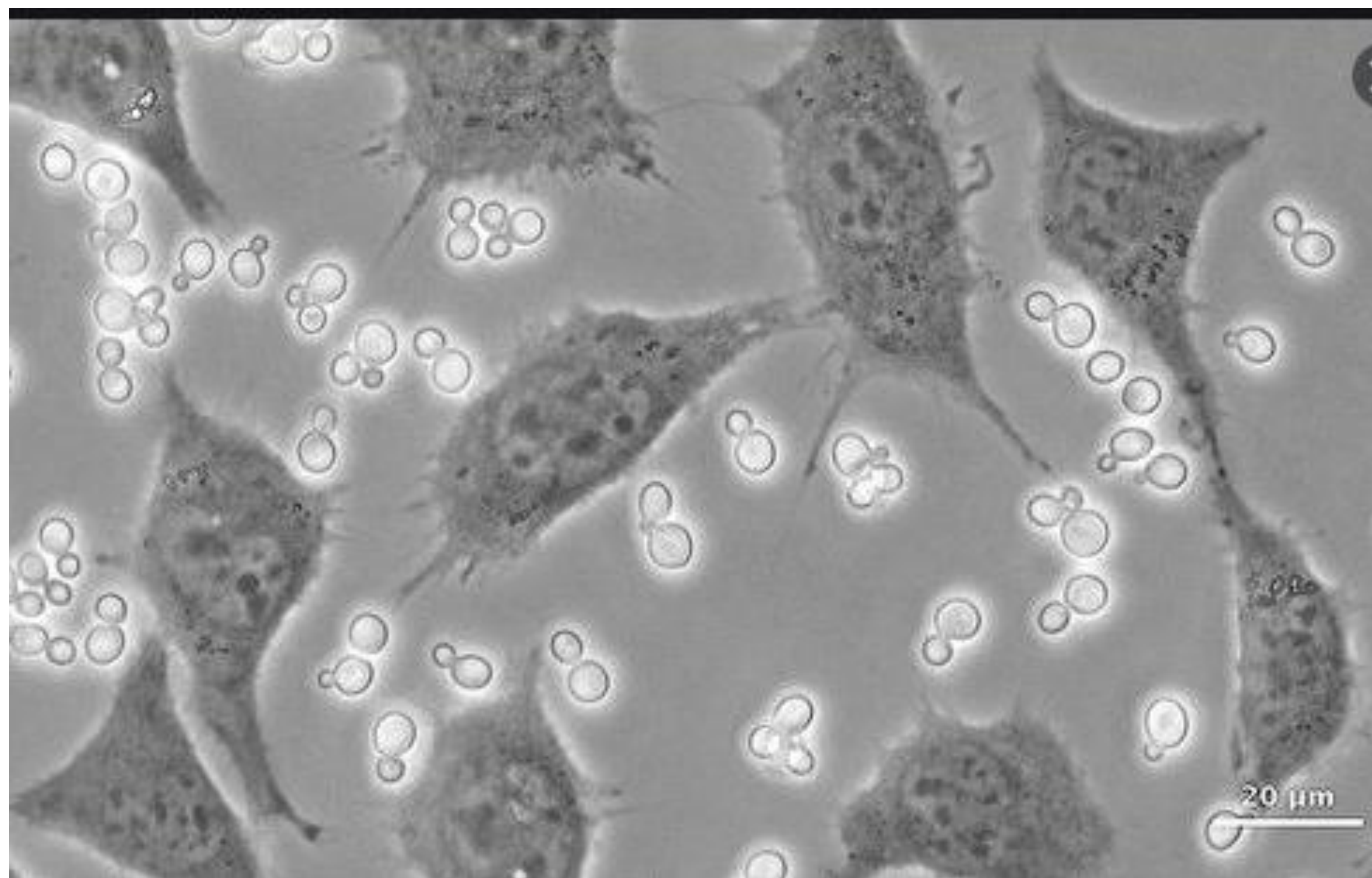
Biological Contamination

- While it is impossible to eliminate contamination entirely, it is possible to reduce its frequency and seriousness by gaining a thorough understanding of their sources and by following good aseptic technique








20 µm

Cryopreservation

If a surplus of cells is available from sub-culturing, they should be treated with the appropriate protective agent (e.g., DMSO or glycerol) and stored at temperatures below –130°C until they are needed. This stores cell stocks and prevents original cell from being lost due to unexpected equipment failure or biological contaminations. When thawing the cells, the frozen tube of cells is warmed quickly in warm water, rinsed with medium and serum and then added into culture containers once suspended in the appropriate media

DMSO: dimethyl sulfoxide

Applications of Cell Line

A. Vaccines Production

One of the most important uses of cell culture is in research and production of vaccines. The ability to grow large amounts of virus in cell culture eventually led to the creation of the polio vaccine, and cells are still used today on a large scale to produce vaccines for many other diseases, like rabies, chicken pox, hepatitis B, and measles. In early times, researchers had to use live animals to grow poliovirus, but due to the development of cell culture technique they were able to achieve much greater control over virus production and on a much larger scale which eventually develop vaccines and various treatments. However, continuous cell lines are not used in virus production for human vaccines as these are derived from malignant tissue or possess malignant characteristics.

B. Virus cultivation and study

Cell culture is widely used for the propagation of viruses as it is convenient, economic, easy to handle compared to other animals. It is easy to observe cytopathic effects and easy to select particular cells on which virus grow as well as to study the infectious cycle. Cell lines are convenient for virus research because cell material is continuously available. Continuous cell lines have been extremely useful in cultivating many viruses previously difficult or impossible to grow.

C. Cellular and molecular biology

Cell culture is one of the major tools used in cellular and molecular biology, providing excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging), the effects of different toxic compounds on the cells, and mutagenesis and carcinogenesis. The major advantage of using cell culture for any of these applications is the consistency and reproducibility of results that can be obtained from using a batch of clonal cells.

D. In Cancer Research

Normal cells can be transformed into cancer cells by methods including radiation, chemicals and viruses. These cells can then be used to study cancer more closely and to test potential new treatments.

E. Gene therapy

Cells having a functional gene can be replaced to cells which are having non-functional gene, and for which the cell culture technique is used.

F. Immunological studies

Cell culture techniques are used to know the working of various immune cells, cytokines, lymphoid cells, and interaction between disease causing agent and host cell.

G. Others

Cell lines are also used in in-vitro fertilization (IVF) technology, recombinant protein and drug selection and improvement.

Preparation of primary Chicken embryo fibroblast

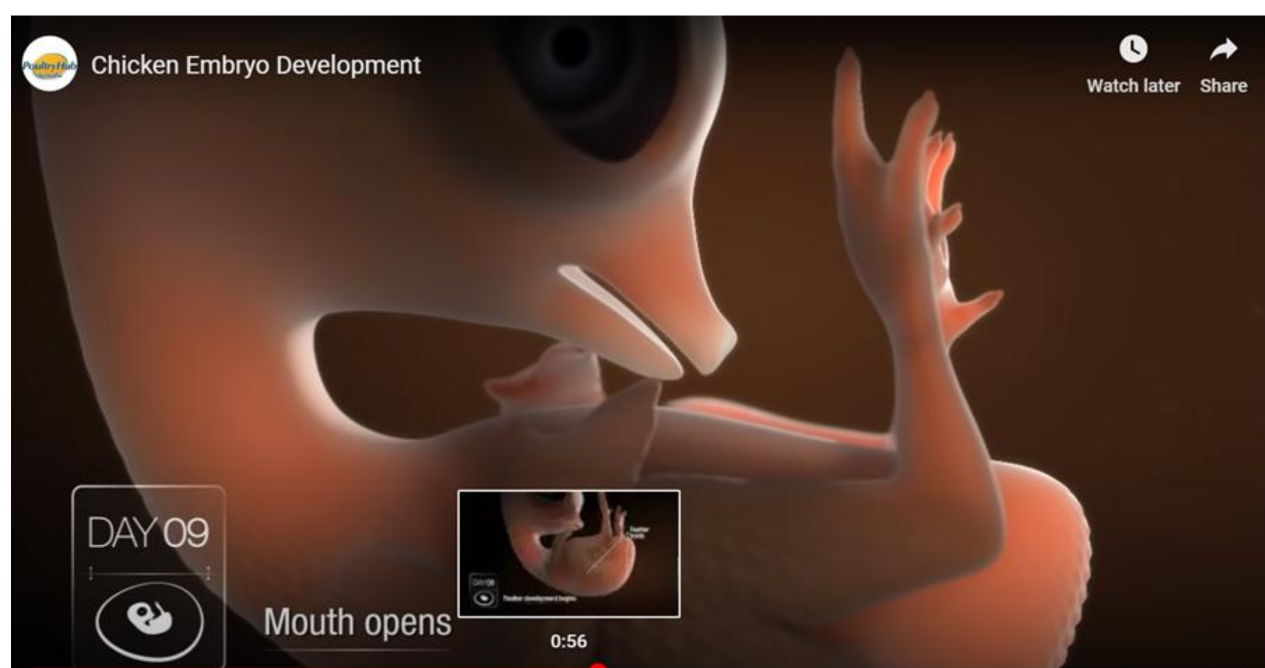
Following the method of (Hichner, 1980; Karel, *et al.*, 1998) to prepare chicken embryo fibroblast by:

(1) Nine-eleven days incubated eggs were chosen for the preparation of chick embryo fibroblasts. The eggs were candled and the air space marked. After disinfection with alcohol 70% the shell over the air space is removed.

(2) The contents of the egg were poured into a petridish and the embryo is taken out of the amnion sac.

(3) The chick embryos were discard from head, limbs, wings, and removed the viscera, washed the other part by PBS to discard from blood.

(4) The Minced tissue scratched to small fragments or pieces and transferred to sterile trypsinizing flask which contain sterile magnetic rod.


Chicken Embryo Development
Watch later
Share
DAY 09
Mouth opens
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Chicken Embryo Development
Watch later
Share
DAY 11
Tail feathers appear
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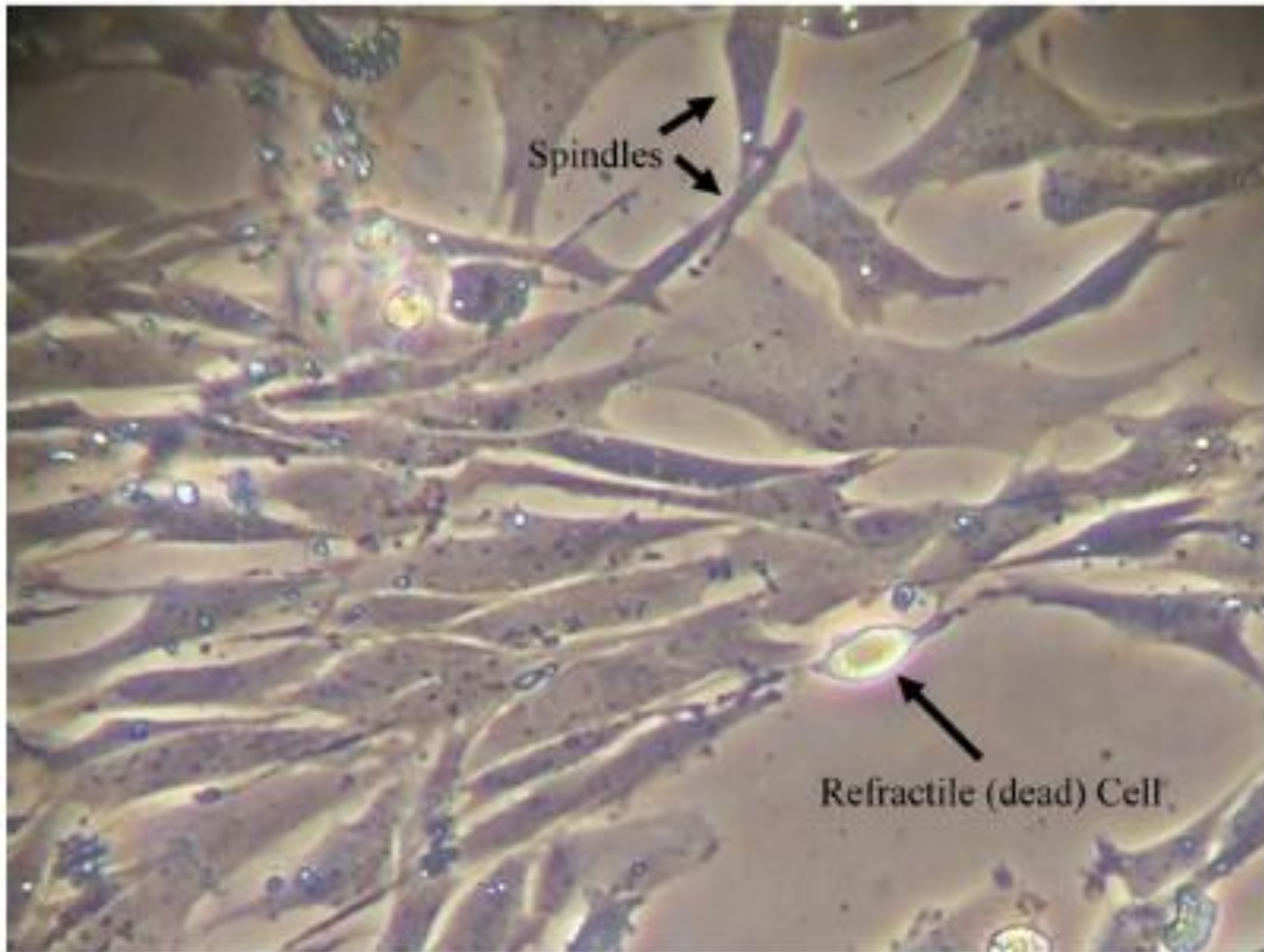
(5) The cells were dispersed by warm Trypsin (0.25% trypsin at 37 °C) and Leaving the trypsinizing flask on magnetic stirrer for 10 minutes.

(6) The cell suspension was filtrated through sterile gauze in to sterile beaker, 20 ml growth media was added to inhibition trypsin activity, the harvested cells Centrifuged at 1200 rpm for 15 min at 4 °C.

(6) Pelleted cells were re-suspended in RPMI growth medium at concentration 1ml of packed cells/200 ml of medium, cells were seeded in 96well plat for TCID50 and infected with ND virus in tissue culture flask for the gene expression .

ND virus: newcastle disease virus

- microscopy of primary chicken embryo fibroblast cells.



- Avian strains of influenza A virus replicate in chick embryo fibroblasts which is important for the study and isolation of these viruses. Investigators have used these cells to assay the infectivity of a strain of H5N2 and test the antiviral properties of several antiviral compounds