Animal Tissue culture

Arnold in 1880, showed that the leucocytes can divide outside the body. Jolly, 1903, demonstrated that many of the animal tissue explants immersed in serum/ lymph or ascetic fluid can divide *in vitro*.

The term tissue culture may be defined as culture of whole organs or tissue fragments or dispersed cells on a suitable nutrient medium and optimum culture conditions.

The tissue culture can be broadly divided into catagories:

1. Cell culture and 2. Organ culture.

1. Cell culture: the individual cells are obtained from spontaneous migration of cells from explants or mechanical or enzymatic disaggregation of tissue explants. These cells are maintained as monolayers or cell suspension during culture.
2. Organ culture: small tissue fragments or whole embryonic organs are cultured in vitro in organ culture and these structures retain these tissue architecture. Organ cultures are of two types
    a. Oganotypic culture: if the tissue explants maintains its original strucuture and function during culture it is called organotypic culture.
    b. Histotypic culture: the cells of the explants that re-associate to form a new three dimensional structure and are not dependent on source form which these are derived. Such type of culture is called histotypic culture.

Another categorization of tissue culture

1. Primary cell culture: cell culture arising directly from differentiated tissue is referred to as primary cell culture. Such cell die oiut after 30 to 50 cell divisions.
2. Cell lines: the progeny cell derived from cells that have become altered from their parental cells such that they acquired a different morphology, grow faster and are able to start a culture from a smaller number of cells. The progeny derived from such exceptional cells have unlimited life. These are designated as cell lines.

The cultivation of mammalian cells from a tissue begins with dissociation of tissue fragments into its component cells by treatment with proteolytic enzyme called trypsin. The disaggregated cells are now placed on a tissue culture flask having a flat surface. Eventually, the flat surface of tissue culture flask would be covered with continuous layer of cells. This layer is only one cell thick and hence referred to as monolayer. The cells of primary culture can be detached from the tissue culture flask surface using trypsin with addition of chelating agent EDTA. The detached and disorganized cells can now be re-cultured into a new tissue culture flask by adding fresh culture media. This cell-culture is now termed as secondary cell culture. These cells multiply at constant rate over successive transfers and such cells comprise a cell strain. Cell strains do not have infinite life and divide only a finite number of times before their growth rate declines and they die after 30 to 50 times (depending on type of tissue).

Transformation

In microbiology, where the term was first used in the context, *transformation implies a change in phenotype that is dependent on the uptake of new genetic material.*

The **transformation of cultured cell** *implies a spontaneous or induced permanent phenotypic change resulting from a heritable change in DNA and gene expression.*

Or alternatively the cell transformation may defined as
The alteration in a culture that give rise to a continuous cell line is referred to as in-vitro transformation.

What is the difference between transformation and immortalization?

Immortalization means the acquisition of an infinite life span and transformation implies an additional alteration in growth characteristics (anchorage independence, loss of contact inhibition and density limitation of growth) that will often, but not necessarily, correlate with tumorigenicity.

The term transformation has been applied to the process of formation of a continous cell line partly because the culture undergoes morphological and kinetic alterations, but also because the formation of a continuous cell line is often accompanied by an increase in tumorigenicity. A number of the properties of continous cell lines such as reduced serum requirement, reduced density limitation of growth, aneuploidy and so on. Similar morphnological and behavioral changes can also be observed in cells that have undergone virally or chemically induced transformation.

The transformation may be achieved through

- Infection with a transforming virus, such as polyoma
- Transfection with mutant tumour causing genes (such as mutant *ras* gene).
- Exposure to ionizing radiations (such as UV rays).
- Chemical carcinogens.
- Somatic cell hybridization. This includes the somatic cell fusion of one normal cell and one cancerous cells. Eg. Formation of hybridoma by fusion of antibody producing B cell and myeloma cell (cancerous cell).

Most of the cell lines have been derived from tumors. The possession of cancerous phenotype allows easier adoption to the growth in the cell culture. That may be due to the activation of telomerase gene and aneuploid nature.
The transformation is associated with genetic instability and three major classes of phenotypic change may be:

1. **Immortalization**: the acquisition of an infinite life span.
2. **Aberrant growth control**: the loss of contact inhibition of cell motility, density-limitation of cell proliferation and anchorage dependence.
3. **Malignancy**: growth of invasive tumors *in vivo* (that is if these cells are injected into an animal, the animal would develop tumor).

How the immortalization is achived?

By the control of senescence: The cancerous cells are heterogenous owing to accumulation of lot of mutation. So, in the population of cells some cells may arise that have active telomerase gene. This gene is active only in germ cells (ova or spermatozoa) and remains inactive in somatic cells. When this gene becomes active, there is enormous production of enzyme telomerase that is required for the replication telomeres (ends of chromosomes). In normal somatic cells as the gene for telomerase remains inactive as a result the enzyme telomerase is not present. So, the replication of telomeres does not occur. Thus after every cell division, the length of telomeres becomes shorter and shorter that contributes their ageing.

However, in cancerous cells owing to replication of telomeres by the enzyme telomerase, the ageing of cells does not occur eventually contributing to the immortality of the cells.

Genetic instability of cell lines: the two main causes of genetic instability of cell lines are (1) spontaneous mutation rate appears to be higher in vitro perhaps owing to high rate of cell proliferation.
(2) Mutant cells are not eliminated unless their growth capacity is impaired.

Media for mammalian cell culture

Four basic criteria for culture medium for animal cells:

1. The medium must provide all nutritional requirements of the cell.
2. The medium must maintain a pH value 7.0 to 7.3, despite the production of acid, i.e. it must be adequately buffered.
3. The medium must be isotonic with cell cytoplasm.
4. Medium must be sterile.

The composition of the growth medium is very crucial and is complex and generally not completely defined (composition of medium is not known) because of presence of serum.

The basic component of the media is a balanced salt solution whose function is to provide:

1. Essential inorganic ions.
2. Correct osmolarity
3. Correct pH.
4. Source of energy in form of glucose or pyruvate.
5. pH indicator (Phenol red).

Basic composition of the media

1. Vitamins: folic acid, inositol, nicotinamide, pyridozal phosphate, riboflavin, thiamine.
2. 5-10% Fetal calf serum (FCS): precise function of FCS is not fully understood however, it is believed to promote attachment of cells to tissue culture flask surface and stimulate cell division. These two basic function of FCS is believed to mediated by certain serum components such as α-globulins and hormones.
   In some cell lines the FCS may be replaced with albumin supplemented with insulin, selenium and transferin.
3. Sodium bicarbonate: maintains pH of the media. This releases the CO_2 into the atmosphere and hydroxyl ions into the medium.
   Alternatively, HEPES buffer may be used instead of sodium bicarbonate.
4. Antibiotics: The most important problem with the animal tissue culture is the occurrence of contamination by bacteria, fungi and mycoplasma. For averting these problems, penicillin is used to avoid Gram negative bacteria, Nystatin for fungal contamination and tetracycline, kanamycin and gentamicin for inhibition of mycoplasma.

Applications of Animal Tissue Culture

1. Production of monoclonal antibodies.

2. Production of recombinant proteins using eukaryotic expression vectors producing proteins such as interferons and lymphokines.
3. Cytotoxicity testing.
4. Propagation of viruses for research and vaccine production.
5. Studying the tissue damaging properties of pathogens.