Lab.7: Animal cell culture

An important aspect of any biotechnological processes is the culture of animal cells in artificial media. These animal cells in culture are used in recombinant DNA technology, genetic manipulations and in a variety of industrial processes.

There are certain terms that are associated with the cell lines:
(i) Split ratio- The divisor of the dilution ratio of a cell culture at subculture.
(ii) Passage number- It is the number of times that the culture has been cultured.
(iii) Generation number- It refers to the number of doublings that a cell population has undergone.

Requirements for Animal cell culture
Following parameters are essential for successful animal cell culture:

1. Temperature- In most of the mammalian cell cultures, the temperature is maintained at 37^0C in the incubators.
2. pH- Most media maintain the pH between 7 and 7.4. A pH below 6.8 inhibits cell growth.
3. Osmolality- A change in osmolality can affect cell growth and function. Salt, Glucose and Amino acids in the growth media determine the osmolality of the medium.
4. Culture media- It should contain chemical constituents which the cells or tissues are incapable of synthesizing. Generally the media is the mixture of inorganic salts and other nutrients capable of sustaining cells in culture such as amino acids, fatty acids, sugars, ions, trace elements, vitamins, cofactors, and ions. Glucose is added as energy source. Phenol Red is added as a pH indicator of the medium.

There are two types of media used for culture of animal cells and tissues:

1) **Natural Media** - They are the natural sources of nutrient sufficient for growth and proliferation of animal cells and tissues. They are fall in three categories;

i) Coagulant, such as plasma clots.
ii) Biological fluids such as serum.
iii) Tissue extracts for example Embryo extracts.

2) Synthetic media - They are prepared artificially by adding several organic and inorganic nutrients, vitamins, salts, serum proteins, carbohydrates, cofactors etc. Synthetic media are of two types- Serum containing media (media containing serum) and serum- free media (media without serum).

Examples of some media are: minimal essential medium (MEM), RPMI 1640 medium, CMRL 1066, F12 etc.

Advantages of serum in culture medium are:

- Serum binds and neutralizes toxins.
- Serum contains a complete set of essential growth factors, hormones, attachment and spreading factors, binding and transport proteins.
- It contains the protease inhibitors.
- It increases the buffering capacity.
- It provides trace elements.

Disadvantages of serum in culture medium are:

- It is not chemically defined and therefore it's composition varies a lot.
- It is sometimes source of contamination by viruses, mycoplasma, prions etc.
- It increases the difficulties and cost of downstream processing.
- It is the most expensive component of the culture medium.

Characterization of cell lines:

The cell lines are characterized by their:

a) Growth Rate - A growth curve consist of:

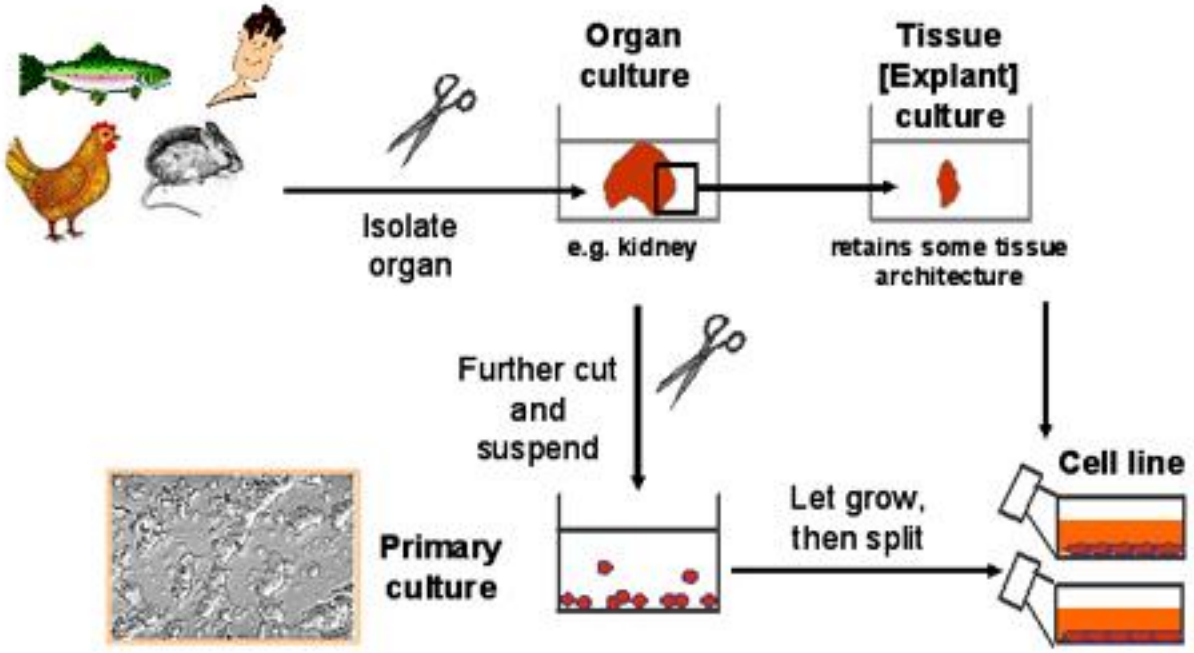
1) Lag Phase: The time the cell population takes to recover from such sub culture, attach to the culture vessel and spread.

2) Log Phase: In this phase the cell number begins to increase exponentially.

3) Plateau Phase: During this phase, the growth rate slows or stops due to exhaustion of growth medium or confluence.

b) Karyotyping - Karyotyping is important as it determines the species of origin and determine the extent of gross chromosomal changes in the line. The cell lines with abnormal karyotype are also used if they continue to perform normal function. Karyotype is affected by the growth conditions used, the way in which the cells are subcultured and whether or not the cells are frozen.

How can a cell line be derived?



Types of in vitro systems