

MICROBIOLOGY....

CULTURE MEDIA

IV SEMESTER MSC ZOOLOGY

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ANIMAL CELL AND TISSUE CULTURE

CULTURE MEDIA :Natural media and Artificial media

CULTURE MEDIA

The nutrient media used for culture of animal cells and tissue

It must be able to support their survival as well as growth i.e.

culture media are divided into two. They are

NATURAL MEDIA

ARTIFICIAL MEDIA

The choice of medium depends mainly on the type of cells to be cultured and the objective of culture (growth, survival, differentiation, etc.)

Non transformed or normal cells and primary cultures from healthy tissues require defined quantities of proteins , growth factors and hormones

Immortalized cells produce most of these factors but may still need some of the growth factors present In the serum

Transformed cells synthesize their own growth factors ., in fact addition of growth factors may even be detrimental in such cases . But even these cultures may require factors like insulin , transferrin lipids etc.

NATURAL MEDIA

These media consist of naturally occurring biological fluids

They are of three types

- ❖ cagula or clots
- ❖ Biological fluids
- ❖ Tissue extracts

Clots

The most commonly used clots are plasma clots ,which have been in use for a long time

Plasma is now commercially available either in liquid or lyophilized state

It may also be prepared in laboratory, usually from the blood of male fowl, but blood clotting must be avoided during the preparation

BIOLOGICAL FLUIDS

various biological fluids used as culture media

e.g ; amniotic fluid ascitic acid and pleural fluid , aqueous humour from eye , insect haemolymph , serum, etc.

Serum is most widely used

Serum is the liquid exuded from coagulating blood

Serum may be obtained from adult human blood, placental cord blood, horse blood or calf blood(foetal calf serum , new born calf serum, and calf serum); Of these

Calf serum and foetal serum are commonly used.
Human serum is sometimes used for human cells

TISSUE EXTRACT

Chick embryo extract is most commonly used tissue extract, but bovine embryo extract is also used.

Other tissue extract that have been used are spleen, liver, bone marrow, leucocytes, etc .

Tissue extract can often be substituted by a mixture of amino acids and certain other organic compounds.

The natural biological fluids are generally used for organ culture

For cell cultures, artificial media with or without serum are used.

Artificial Media

Different artificial media have been devised to serve one the following purposes

- Immediate survival
- Prolonged survival
- Indefinite growth
- Specialized functions

The various artificial media developed for cell culture may be grouped in to four classes :

- Serum containing media
- Serum- free media
- Chemically defined media

SERUM CONTAINING MEDIA

The various defined media, e.g. Eagle's minimum essential medium etc (serum free media), when supplemented with 5-20% serum are good nutrient media for culture of most types of cells

Serum quality is tested by the manufacturer before it is supplied

Serum is heat inactivated (30 min at 56°C) primarily to inactivate the complement system

The serum provides various plasma protein, peptide, lipids, carbohydrates, minerals, and some enzymes.

The serum serves the following major functions

- It provides the basic nutrients for cells.

- It provides several hormones e.g insulin
- It contain several growth factors e.g platelet – derived growth factor(PDGF),epidermal growth factor e.t.c.
- It provides several binding proteins e.g. Albumin, transferrin
- It provides proteins e.g. Fibronectin
- It increases viscosity of medium and thereby , protects cells from mechanical damages, e.g. Shear forces during agitations of suspension cultures
- It provides several minerals e.g. Na, K, Fe , Zn e.t.c.
- Protease inhibitors present in serum protect cells ,especially trypsinised cells, from proteolysis
- It also act as a buffer

Disadvantages

- May inhibit growth of some cell types e.g. Epidermal keratinocytes
- It may contain some cytotoxic or potentially cytotoxic constituents
- There is a large variation in serum quality from one batch from to another, this requires costly and time consuming testing every time a new batch of serum has to be used
- Some growth factors may be inadequate for specific cell types and may need supplementation
- It interferes with downstream processing
- The supply of serum is always lower than demand

SERUM –FREE MEDIA

Many approaches be used to overcome the disadvantages of serum contain media. It includes

- Analytical approach based o the analysis of serum constituents
- Synthetic approach to supplement basal media by various combinations of growth factors
- Limiting factor approach

ADVENTAGES

- Improved reproductivity of results
- Easier downstream processing of products from cultured cells
- Toxic effects of serum are avoided
- Bioassays are free from interference due to serum proteins
- There is no danger of degradation of sensitive proteins by serum proteases
- They permit selective culture of differentiated and producing cell types from the heterogeneous cultures

DISADVANTAGES

- Most serum –free media are specific to one cell type
- Reliable serum – free preparations, for most of the formulations
- are not available commercially
- A greater control of pH , temperature , e.t.c , is necessary as compared to that with serum containing media.
- Growth rate and the maximum cell density attained are lower than those with serum containing media.
- Cells tend to become fragile during prolonged agitated cultures unless biopolymers or synthetic polymers are added.

Several defined media have been evolved from the eagle's minimal essential medium (MEM), e.g. Dulbecco's enriched modification (DME), HAM'S F12, CMRL1066, RPMI1640, McCOY'S 5A and Iscove's modified Dulbecco's (IMDM): all are commercially available