FACULTY OF ENGINEERING & TECHNOLOGY

LT.5 Culture media and role of serum

Outline

1. Natural Media
2. Synthetic Media
3. Categories of artificial media
4. Components of media
5. Serum
6. Advantages and disadvantages of serum

Media & its role in cell culture

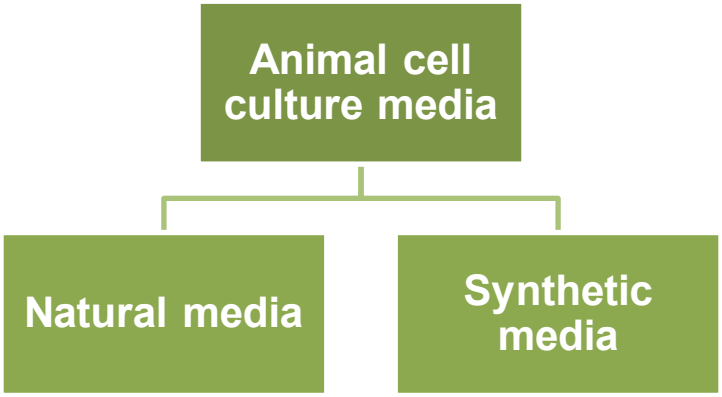
The role of media is to provide all nutritional requirements needed by growing cells in the culture.

It is used for in vitro maintenance and propagation of animal cells.

Media plays following role during cell culture:

- Nutritional requirements such as energy source, pH, vitamins , amino acids, trace elements etc.
- Protects cells from shear damage during growth in bioreactors

❖Animal cells can be cultured either using a completely natural medium or an artificial/synthetic medium along with some natural products.



•Natural media are derived from biological fluids whereas artificial media are prepared in lab using organic and inorganic components and salts.

Natural Media & Artificial media

Natural media:

Plasma clot, serum, aminiotic fluid are example of natural media. These are isolated from biological fluids.

Type of natural media:

i. Coagulans or plasma e.g. plasma clots

ii. Biological fluids e.g. plasma, serum, lymph, amniotic fluids

iii. Tissue extract e.g. Extract of liver, spleen, tumors, leucocytes and bone marrow, extract of the bovine embryo and chick embryo

Artificial media

- Artificial or synthetic media are prepared by adding nutrients (both organic and inorganic), vitamins, salts, O_2 and CO_2 gas phases, serum proteins, carbohydrates, cofactors.
- The artificial culture media primarily consist of balanced salt solution (BSS) which provide essential inorganic ions, correct osmolarity, required pH (7.0-7.3), energy (= glucose) and a pH indicator (such as phenol red).

Role / Purpose of artificial media

Artificial media serves following purpose:

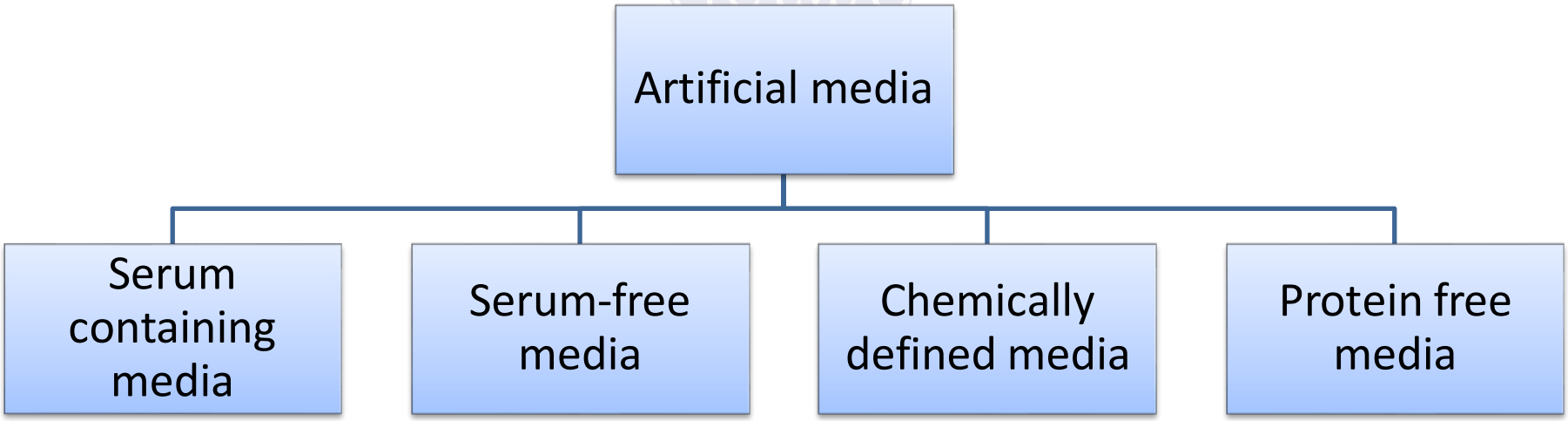
a. Immediate survival (a balanced salt solution, with specific pH and osmotic pressure)

b. Prolonged survival (a balanced salt solution supplemented with various formulations of organic compounds and/or serum)

a. Indefinite growth

b. Specialized function

Categories of artificial media



Serum containing media:

- Serum provides carriers or chelators for labile or water-insoluble nutrients, hormones and growth factors, protease inhibitors, and binds and neutralizes toxic moieties.
- It is used as a low-cost supplement to provide an optimal culture medium

e.g. Fetal Bovine serum

Serum Free media:

These types of media do not contain serum and is specifically formulated to support the culture of a single cell type. E.g. Knockout DMEM, mTESR1 developed for growth of stem cells.

Property of Knockout DMEM

•A medium with lower osmotic pressure than DMEM and an added serum substitute containing animal-source components (KSR), it is for use with mouse embryonic stem cells. The cultures require feeder cells.

Chemically defined media

Chemically-defined media contains components that are all known. These media contain contamination-free ultra pure inorganic and organic ingredients, and. may also contain pure protein additives, like growth factors

Protein free media

It does not contains any protein and only contains non-protein constituents.

e.g. MEM, RPMI-1640 MEM, RPMI-1640

Role

•Promotes superior cell growth

•Superior protein expression

•Easy Downstream processing

Basic component of media

Components	Function	Example
Buffering system	Regulation of pH	Natural buffering: gaseous CO_2 balances with the CO_3/HCO_3
		Chemical Buffering: HEPES, has a superior buffering capacity in the pH range 7.2-7.4 and does not require a controlled gaseous atmosphere .
pH Indicator	Detection of pH variation	Phenol red
Inorganic salts	•Retains osmotic balance. •Regulation of membrane potential	sodium, potassium, and calcium ions
Amino acids	For synthesis of proteins Proliferation of cells	L- Glutamine. It provides nitrogen for NAD, NADPH and nucleotides and serves as a secondary energy source for metabolism.
Carbohydrates	Energy source	glucose and galactose, however, some contain maltose and fructose.
Fatty acids and lipids	They are particularly important in serum-free media as they are generally present in serum.	

Proteins and peptides	Albumin binds water, salts, free fatty acids, hormones, and vitamins, and transport them between tissues and cells.	albumin, transferrin, and fibronectin
Vitamins	Essential for growth and proliferation of cells.	Essential for growth and proliferation of cells.
Trace elements (required in serum free media)	•Needed in minute amount. •These micronutrients are essential for many biological processes, e.g., the maintenance of the functionality of enzymes.	copper, zinc, selenium and tricarboxylic acid intermediates

Media supplements

•Certain cell lines requires additional nutrient for their growth apart from normal media constituents or these components are not present in media altogether.

•supplements like hormones, growth factors and signaling substances are required for normal growth of some cell lines.

Serum

•It is a clear, slightly viscous fluid obtained after coagulation of blood.

•Serum is a complex mix of albumins, growth factors and growth inhibitors.

•serves as a source for amino acids, proteins, vitamins (particularly fat-soluble vitamins such as A, D, E, and K), carbohydrates, lipids, hormones, growth factors, minerals, and trace elements

Role of serum in media

a. Serum provides the basic nutrients (both in the solution as well as bound to the proteins) for cells.
b. Serum provides several growth factors and hormones involved in growth promotion and specialized cell function.
c. It provides several binding proteins like albumin, transferrin, which can carry other molecules into the cell. For example: albumin carries lipids, vitamins, hormones, etc. into cells.
d. It also supplies proteins, like fibronectin, which promote the attachment of cells to the substrate. It also provides spreading factors that help the cells to spread out before they begin to divide.
e. It provides protease inhibitors which protect cells from proteolysis.
f. It also provides minerals, like Na+, K+, Zn2+, Fe2+, etc.
g. It increases the viscosity of the medium and thus, protects cells from mechanical damages during agitation of suspension cultures.
h. It also acts a buffer.

Advantages & Disadvantages of Serum

Advantages

- **Stimulates cell growth and functions**
- Helps in attachment and spreading of cells
- Acts as buffering agent
- Increases viscosity of medium and minimizes mechanical damage to cells

Disadvantages

- **Batch to batch variability**
- May contains growth inhibiting substance
- High risk of contaminations with virus, fungi, mycoplasma
- Presence of serum pose difficulty during cell culture product recovery from media

Test your Understanding

1. Serum is an example of
    a. Natural media
    b. Synthetic media
    c. Chemically defined media
    d. None of the above
2. Which of the following amino acids are used both as carbon as well as Nitrogen donor for protein
    a. L-Alanine
    b. D-Glycine
    c. L-Glutamine
    d. Y-Aspartic acid
3. Example of tissue extract
    a. Plasma clot
    b. Aminiotic fluid
    c. Extract of liver
4. At what percentage is CO_2 used for buffering?
    a. 5-10%
    b. 11-20%
    c. Any concentration can be used
    d. None of the above
5. Vitamins are essential for groth and proliferation. (True/ False)
6. Batch to batch variability is not a disadvantage of serum (True/ False)
7. mTESR1 media is suitable for growth of…….cells

Reference & Suggested reading

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