Culture Media for animal cells

JA Negrón, Ph.D.

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Summary

- Introduction
- Basal media
- Water, Buffer
- Antibiotics
- Serum
- Chemically defined serum-free media
- Growth factors

Introduction

- > 50 years
- Initially
  - Biological fluids
    - Blood
    - Serum
    - Tissue extract
    - Chemical analysis of biol. Fluids
- Eagle's Minimum Essential Medium (EMEM)

Mammalian cell Culture media basic requirements

- Type used depends on the cell type and whether primary or secondary cultures or infinite cell lines
- Requirements
  - ions; Na, K, Ca, Mg, Cl, P, bicarbonate
  - Trace elements; iron, zinc, selenium
  - Sugars such as glucose
  - Amino acids
  - Serum; contains hormones, growth factors to promote proper growth
- Also; antibiotics, not required for growth, but used to control the growth of bacterial contaminants.
- Also; many have a pH indicator, phenol red

Basal Media

- Undefined media
  - Batch variation plus vulnerability to contamination
- Simple basal media
  - BME (Eagle's Basal Media)
  - EMEM (Eagle's Minimum Essential Media)
  - GMEM (Glasgow's Modification of Eagle's medium)
  - DMEM (Dulbecco's Modification of Eagle's Medium)
  - RPMI (Roswell Park Memorial Institute medium)

Eagle's Basal media (BME)

- Originally designed to grow mouse-L and HeLa cells
- Is one of the most widely used of all synthetic cell culture media.
- To measure the growth response of normal (WI-38) and transformed (mouse and HeLa) cells in monolayer culture.
- BME is the predecessor of Eagle's Minimum Essential Medium (EMEM or MEM) and Dulbecco's Modified Eagle's Medium (DME).

Eagle's Minimum Essential Medium (EMEM)

- 13 amino acids
- 8 vitamins
- 6 ionic species
- Dialyzed serum
  - To provide the necessary undefined components

The Essential Amino Acids
Histidine
Isoleucine
Leucine
Lysine
Methionine (and/or cysteine)
Phenylalanine (and/or tyrosine)
Threonine
Tryptophan
Valine

Glasgow's Modification of Eagle's medium

- Was originally developed by Ian MacPherson and Michael Stoker as a modification of Eagle's medium.
- This medium was used to study the genetic factors affecting cell competence. The polyoma virus was used to transform four fibroblast clones from a culture of baby hamster kidney cells.
- The medium was developed by modifying Eagle's BME by adding 10% tryptose phosphate and twice the normal concentration of amino acids and vitamins.

Dulbecco's Modified Eagle's medium (DMEM)

- Dulbecco's Modified Eagle's medium (DMEM) is among the most widely used modifications of Eagle's Medium.
- DMEM is a modification of Basal Medium Eagle (BME) that contains a four-fold higher concentration of amino acids and vitamins, as well as additional supplementary components.
- The original DMEM formula contains 1000 mg/L of glucose and was first reported for culturing embryonic mouse cells.
- A further alteration with 4500 mg/L glucose has proved to be optimal for cultivation of certain cell types.

Roswell Park Memorial Institute medium (RPMI)

- Developed by Moore et al., at Roswell Park Memorial Institute, hence the acronym RPMI.
- The formulation is based on the RPMI-1630 series of media utilizing a bicarbonate buffering system and alterations in the amounts of amino acids and vitamins.
- RPMI-1640 medium has been used for the culture of human normal and neoplastic leukocytes.
- RPMI-1640 when properly supplemented, has demonstrated wide applicability for supporting growth of many types of cell cultures, including fresh human lymphocytes in the 72-hour phytohemagglutinin (PHA) stimulation assay.

Water

- The quality of water is critical.
  - Overall process of water purification
    - Reverse osmosis or distillation (remove major chemicals)
    - Charcoal filtration (↓organic and inorganic impurities)
    - Deionization (↓trace metals or ions)
    - Micropore filtration (↓microbial contamination)
  - Evaluation of water purity
    - Electrical resitance in MΩcm
      - The less dissolved inorganic salts, the higher resistance and the purer the water.
    - Standard: ≥ 20 MΩcm at 25◦C

Buffer

- An aqueous solution consisting of a mixture of a weak acid and its conjugate base or a weak base and its conjugate acid.
- The extent of ionization of a weak acid (the pK_a) influences the final concentration of H^+ ions (the pH) of the solution, there must be a relationship between pH and the pK_a of a weak acid. This relationship is given by the **Henderson–Hasselbalch equation**:

$$pH = pK_a + \frac{[base]}{[acid]}$$

Effective pH range for buffers = pKa ± 1

Buffer

- Bicarbonate (HCO_3^-)is often included to act as a buffer system in conjunction with carbon dioxide (CO_2) environment (5-10%) in which the cells should be cultured.
- Organic buffer HEPES (pH 7.0) is usually added to the medium

$$HCO_3^- + H^+ \rightleftarrows H_2CO_3 \rightleftarrows HCO_3^- + OH^- \rightleftarrows CO_2 + H_2O$$

From liquid medium

From gas cylinder with CO_2 /air

pH = 6.9-7.4

Antibiotics

- Often included in media for short term cultures.
- Extensive use of antibiotics may cause the selective retention of antibiotic-resistant contaminants in the laboratory.
- When used, a cocktail is recommended:
  - Penicillin (against gram-positive bacteria)
  - Streptomycin (against gram-negative bacteria)
  - Amphotericin-B (antifungal)

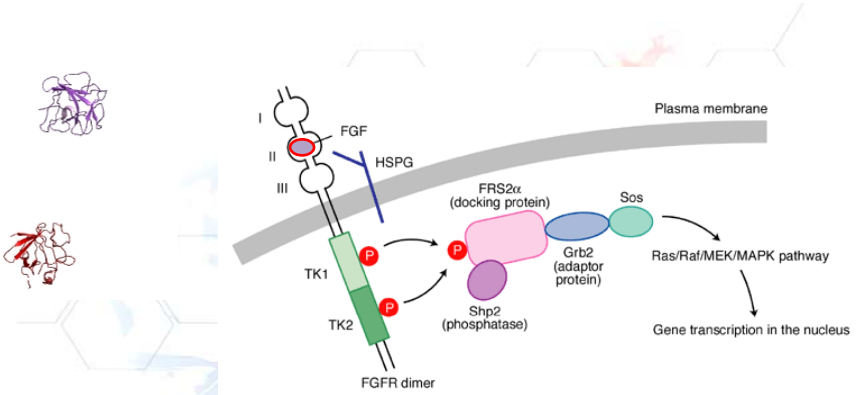
Growth Factors

- Proteins added to the cell culture media in order to enhance cell growth (specially important in the absence of serum or reduced serum formulation).
- Mitogenic polypeptides
  - Fibroblast growth factor or endothelian (aFGF, bFGF)
  - Insulin-like growth factor (IGF)
  - Epithelial growth factor (EGF)
  - Nerve growth factor (NGF)
  - Platelet-derived growth factor (PDGF)
  - Transforming growth factor (α- TGF, β-TGF)

FIBROBLAST GROWTH FACTOR (FGF)

- The name "fibroblast growth factor" (FGF) is a limiting description for this family of cytokines.
- Induce fibroblast proliferation, the original FGF molecule (FGF-2 or FGF basic) is now known to also induce proliferation of endothelial cells, chondrocytes, smooth muscle cells, melanocytes, as well as other cells.
- The FGF superfamily consists of 23 members, all of which contain a conserved 120 amino acid (aa) core region that contains six identical, interspersed amino acids. The superfamily members act extracellularly through four tyrosine kinase FGF receptors, with multiple specificities noted for almost all FGFs.
- Play substantial roles in development, angiogenesis, hematopoiesis, and tumorigenesis

Fibroblast Growth factor

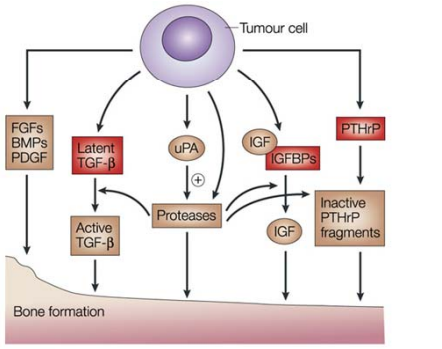


Structure and function of fibroblast growth factor receptors (FGFRs)

Expert Reviews in Molecular Medicine©2003 Cambridge University Press

Model for osteoblastic bone metastases caused by prostate cancer

- The production of factors such as fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF) and transforming growth factor- (TGF-) by tumour cells can directly stimulate osteoblast activity and subsequent bone formation during prostate cancer.



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Nature Reviews Cancer **2**, 584-593 (August 2002)

Chemically defined serum-free media

- The use of serum in culture media is associated with several problems:
  - Batch to batch variation
  - High protein content (not good for protein purification)
  - Risk of contaminants (like viruses, micoplasma, and prions)
  - Risk of transmission of contaminants to humans
  - Interfering with the hormones and growth factors effects
  - Limited availability
  - High cost
- It is becoming undesirable for large scale process in industry

Chemically defined serum-free media

- Distinction between Serum Free Media and Chemically Defined Media (all components and concentrations are known).
- Many advantages:
  - Cells may show enhanced growth characteristics (like differentiation) due to hormones and/or growth factors
  - It is possible to select specific cell types from mixed populations
  - Possibility of studies of the interaction of physiological regulators or drugs

REFERENCES

- Eagle, H. 1955. Nutrition Needs of Mammalian Cells in Tissue Culture. Science. 122: 501-504.