Cell culture

Animal cell culture can be described as in vitro maintenance and propagation of animal cells using a suitable nutrient media. Culturing is a process of growing animal cells artificially.

The nutrient media used for culture of animal cells and tissues must be able to support their survival as well as growth, *i.e.*, must provide nutritional, hormonal and stromal factors. The various types of media used for tissue culture may be grouped into two broad categories:

1. **Natural Media**
2. **Artificial Media**

Basic Components in the Culture Media

1. Energy sources: Glucose, Fructose, Amino acids
2. Nitrogen sources: Amino acids
3. Vitamins: Generally water soluble vitamines B & C
4. Inorganic salts: Na+, K+, Ca2+, Mg2+
5. Fat and Fat soluble components: Fatty acids, cholesterols

6. Nucleic acid precursors
7. Antibiotics
8. Growth factors and hormones
9. pH and bufferig systems
10. Oxygen and CO2 concentration.

Animal cell culture media vary in their complexity but most contain:

- Amino acids 0.1-0.2 mM
- Vitamins 1 µM
- Salts NaCl 150 mM
- KCl 4-6 mM
- CaCl 1 mM
- Glucose 5-10 mM

Natural Media

These media consist solely of naturally occurring biological fluids and are of the following three types:

1. Cagula or clots
2. Biological fluids
3. Tissue extracts.

The natural biological fluids are generally used for organ culture. For cell cultures, artificial media with or without serum are used.

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 the laboratory, usually from the blood of male fowl, but blood clotting must be avoided during the preparation.

Biological Fluids

Serum is the most widely used. It contains various amino acids, hormones, lipids, vitamins, polyamines, and salts containing ions such as calcium, ferrous, ferric, potassium etc. It also contains the growth factors which promotes cell proliferation, cell attachment and adhesion factors.

Serum may be obtained from adult human blood, placental cord blood, horse blood or calf blood (foetal calf serum, newborn calf serum, and calf serum); of these foetal calf serum is the most commonly used.

Tissue Extracts

Tissue or organ extracts and/or hydrolysates (e.g., bovine pituitary extract (BPE), bovine brain extract, chick embryo extract and bovine embryo extract), and animal-derived lipids and fatty acids, peptones, sterols (e.g., cholesterol) and lipoproteins (e.g., high-density and low-density lipoproteins (HDLs and LDLs, respectively) are used in culturing of animal cells.

Tissue extracts for example, Embryo extracts— Other biological fluids used as natural media include amniotic fluids, ascetic and pleural fluids, aqueous humour (from eye), serum ultra filtrate, insect haemolymph etc.

Chick embryo extract is the most commonly used tissue extract, but bovine embryo extract is also used. Other tissue extracts that have been used are spleen, liver, bone marrow, etc. extracts. Tissue extracts can often be substituted by a mixture of amino acids and certain other organic compounds.

Artificial Media

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

1. Immediate survival (a balanced salt solution, with specified pH and osmotic pressure is adequate),
2. Prolonged survival (a balanced salt solution supplemented with serum, or with suitable formulation of organic compounds),
3. Indefinite growth
4. Specialized functions.

The various artificial media developed for cell cultures may be grouped into the following four classes:

(i) Serum containing media

(ii) Serum free media

(iii) Chemically defined media

(iv) Protein free media.

SERUM

Liquid yellowish, clear content left over after fibrin and cells are removed from the blood is known as serum. Calf (bovine), foetal bovine, or horse are used, in some cases human.

Fetal bovine serum (FBS) (10-20% v/v) is the most commonly applied supplement in animal cell culture media. Normal growth media often contain 2-10% of serum.

Thus, serum and/or animal extracts are commonly used as relatively low-cost supplements to provide an optimal culture medium for the cultivation of animal cells.

Serum Contents

Proteins and Polypeptides 40-80 mg/ml
Albumin 20-50 mg/ml
Fetuin 10-20 mg/ml
Fibroncetin 1-10 µg/ml
Globulins 1-15 µg/ml
Protease inhibitors (α1-anti-trypsin) 0.5-2.5 mg/ml
Transferrin 2-4 mg/ml
Amino Acids 0.01-1.0 µM
Lipids 2-10 mg/ml
Cholesterol 10 µM

Fatty acids 0.1–1.0 µM

Linoleic acids 0.01-0.1 µM

Phospholipids 0.7-3.0 mg/ml

Carbohydrate 1.0-2.0 mg/ml

Glucose 0.6-1.2 mg/ml

Hexosamine 0.6-1.2 mg/ml

Lactic acid 0.5-2.0 mg/m

Pyruvic acid 2-10 µg/ml

Polyamines 0.1-1.0 µM

Putrescine, Spermidine

Urea 170-300 µg/ml

Inorganics 0.14-0.16 M

Ca, Cl, Fe, K, P, Se, Na

Hormones 0.1-200 nM

Hydrocortisone 10-200 nM

Insulin 1-100 ng/ml

Triiodothyronine 20 nM

Thyroxine 100 nM

Vitamins 10 ng-10 µg/ml

Vitamin A 10-100 ng/ml

Folate 5-2 ng/ml

Chemically Defined Media:

These media contain contamination free ultra pure inorganic and organic constituents, and may contain pure protein additives, like insulin, epidermal growth factor, etc. that have been produced in bacteria or yeast by genetic engineering with the addition of vitamins, cholesterol, fatty acids and specific amino acids.

Synthetic media

Synthetic media are prepared artificially by adding several nutrients*(orgtmie* and inorganic), vitamins, salts, O_2 and CO_2 gas phases, serum proteins, carbohydrates, cofactors, etc. However, different types of synthetic media may be prepared for a variety of cells and tissues to be cultured. It can be prepared for different functions. Basically, synthetic media are of two types, *serum-containing media (i.e.* the media containing serum) and *serum-free media (i.e.* media devoid of serum).

Example of some of the media are: minimal essential medium (MEM) (Eagle, 1955), 199 (Morgan *et al.* 1950), CMRL 1066 (Parker *et al,* 1957), RPMI 1640 (Moore *et al,* 1967) and F12 (Ham, 1965).

Chemical composition of different media (with serum) used for Animal cell and tissue culture (quantities are in mg/l).

	Chemical constituents	*Eagle's MEM*	*Dulbecco's modification*	*Ham's F12*
1.	Amino Acids			
	L-asparagine	126	84	211
	L-cystine	24	48	-
	L-glutamine	292	584	146
	Glycine	-	30	7.5
	L-histidine HC1.H_2O	42	42	21
	L-isoleucine	52	42	21
	L-leucine	52	105	13.1
	L-lysine.HCl	73.1	146	36.5
	L-methionine	15	30	4.48
	L-phenylalanine	33	66	4.96
	L-proline	-	-	34.5
	L-serine	-	42	10.5
	L-threonine	48	95	11.9
	L-tryptophan	10	16	2.04
	L-tyrosine	36	72	5.4
	L-valine	47	94	11.7

	Chemical constituents	*Eagle's MEM*	*Dulbecco's modification*	*Ham's F12*
2.	**Vitamins**			
	Biotin	**-**	**-**	**0.0073**
	D-Ca-pantothenate	**1**	**4**	**0.48**
	Choline chloride	**1**	**4**	**14**
	Folic acid	**1**	**4**	**1.3**
	Inositol	**2**	**7.2**	**18**
	Nicotinamide	**1**	**4**	**0.04**
	Pyridoxal.HCl	**1**	**4**	**0.062**
	Riboflavin	**0.1**	**0.4**	**0.038**
	Thiamine.HCl	**-**	**-**	**1.36**
	Vitamin B12	**-**	**-**	**1.36**
	Pyridoxin.HCl	**-**	**-**	**0.062**

	Chemical constituents	*Eagle's MEM*	*Dulbecco's modification*	*Ham's F12*
3.	Inorganic Salts			
	$CaCl_2$ (anhydrous)	200	200	-
	$CaCl_2.2H_2O$	-	-	44
	$Fe(NO_3)_3.9H_2O$	-	0.1	-
	KC1	400	400	221
	$MgCl_2.6H_2O$	-	-	122
	$MgSO_4.7H_2O$	200	200	-
	NaCl	6800	6400	7599
	$NaHCO_3$	2200	3700	1176
	$Na_2H_2PO_4.H_2O$	140	125	-
	$Na_2HPO_4.7H_2O$	-	-	268
	$CuSO_4.5H_2O$	-	-	0.00249
	$FeSO_4.7H_2O$	-	-	0.834
	$ZnSO_4.7H_2O$	-	-	0.863
	Thymidine	-	-	0.73

	Chemical constituents	*Eagle's MEM*	*Dulbecco's modification*	*Ham's F12*
4.	**Other Chemicals**			
	D-glucose	**1000**	**4500**	**1802**
	Lipoic acid	**-**	**-**	**0.21**
	Phenol red	**10**	**15**	**12**
	Sodium pyruvate	**-**	**110**	**110**
	Hypoxanthine	**-**	**-**	**4.1**
	Linoleic acid	**-**	**0.084**	**-**
	Putrescine.2HCl	**-**	**-**	**0.161**
	Thymidine	**-**	**-**	**0.73**
5.	**CO_2 (gas phase)**	**5%**	**10%**	**5%**

Source : Freshney (1987).

Serum-free media

When the synthetic media are devoid of serum in culture medium, it is called serum-free media. By doing so the medium could be made selective for a particular type of cells because each type of cells requires different chemical constituents and physical factors. Serum-free media should not be used commonly until cheap and better serum-free media are available.

Serum itself has several disadvantages as given below: ***(i)*** it deteriorates within a year and differs with batches, ***(ii)*** a number of batches are required if more than one cell types are used which make difficult for maintaining and co-culturing of cells difficult, ***(iii)*** supply of serum is less than its demand, therefore, medium becomes several times costly, and ***(iv)*** undesirable growth stimulation and inhibition may occur.

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Serum-free medium for certain cell and cell lines.
***Source :* based on Freshney (1987).**

	Serum	*Serum-free medium*	*Cell or cell lines*
1.	CS	MCDB 202	Chick embryofibroblasts
		CMRL 1066	Continuous cell line
		MCDB 110, 202	Fibroblasts, human diploid fibroblasts
		MCDB 402	Fibroblasts, mouse embryofibroblasts, 3T3 cell
2.	FB	MCDB 130	Endothelium
		F12	Skeletal muscles
		HoS	Mouse leukemia, mouse erythroleukemia, skeletal muscles

Advantages and disadvantages of serum in culture media.

Advantages

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

Disadvantages

- It is not chemically defined and, therefore, it is of variable composition lot to lot. It may be a source of contamination by viruses, mycoplasma, prions, etc.Its components may bind , inactivate, antagonise or mimic the action of added medium ingredients.It increases difficulties and cost of down stream processing.It is most expensive ingredient of the culture media
- *Source :* based on Fiechter (1996).