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In Vitro: Nourishment of cells through Ayurvedic perspective

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Abstract: Ayurveda is a traditional system of medicine native to Indian. The dietary practices in Ayurveda encourages a conscious way of living. Nutrition is the combination of catabolism and anabolism of food in the body. Ayurveda explains that your dietary needs and your digest are affected by the rhythms of nature and changes that occur in life. The foundation of Ayurveda nutrition is based on the idea that you are the result of what, when, where, how and why you eat. Nutrition is the science that interprets the interaction of nutrients and other substances in food in relation to maintenance, growth, reproduction, health and disease of an organism. It includes food intake, absorption, assimilation, biosynthesis, catabolism and excretion. Food taken in proper quantity provides strength, vigor, good complexion and nurtures the health of the tissues. Cell culture is the process by which prokaryotic, eukaryotic or plant cells are grown under controlled conditions. In this process culture of cells derived from animal cells. The study of nutrition is gradually concerned with metabolism and metabolic pathways. The sequence of biochemical steps through which substances in human body is different from cell culture nourishment. This article gives basically study of nutrition process in cell culture over Ayurveda outlook.

Keywords: In vitro, nutrition of cells, Ayurveda nourishment of tissues.

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Introduction:

Ayurveda is widely considered to be one of the oldest health care traditions still in practice today. The human body is the product of food and it is considered as the sustainer of life. *Ahara*, *nidra* and *brahmacharya* are recognized as three *upastambhas* essential for the smooth running of life^[1]. Among the three *upastambhas*, *ahara* is considered as best sustainer of life^[2]. *Ahara* is basic individual for the proper nutrition. According to Ayurveda, food and herbs are categorized by their taste, general qualities and how they affect the elemental *doshas* of *vata*, *pitta* and *kapha*^[3].d

Ayurveda is a way of embracing food as life giving energy. *Sushruta* described the body as well as foods are mainly constituted by five *mahabhutas*. A balanced diet in Ayurveda is planned in relation to the known *panchbhautic* composition and the *tridoshic* impacts in living body. Nutrition science investigates the metabolic and physiological responses of the body to diet. The foundation of Ayurveda nutrition is that your food should be eaten mindfully and with gratitude and that it must be fresh of the higher quality, digestible, delicious, lovingly prepared and satisfying to your senses^[4]. Because body is product of food and food is harbinger of happiness and suffering. Proper digestion process is

essential for the growth and nutrition of body. Digestion begins with the production of enzymatic saliva in mouth and sensory organ stimulates this mouth-watering process. When use a variety of food and spices that use flavorful, aromatic and visually appealing with an assortment of colors and textures, it provides with a wide range of nutrients. The Ayurveda approach to food and dietetics is very different from conventional western approach.

Cell culture is the maintenance and growth of the cells of multicellular organisms outside the body in specially designed containers and under precise condition of temperature, humidity, nutrition and freedom from contamination. Cell culturing technology is currently playing a major role in studying basic cell biology, interactive between disease causing agents and cells, effects of drugs on cells, process and triggering of aging and nutritional studies^[5].

Current nutritional knowledge lays emphasis on macronutrient quality of food like protein, carbohydrate and lipids. The bioavailability of these nutrients depends on the physical properties of food matrix. Cell culture is the technique of tissue, cells or organs from an animal and their placement into an artificial environment suitable to their survival and proliferation. Nutrition supplement is necessary for the

proper growth of cells. In artificial environment, nourishment of cells is unique and different process. This article provides information about the nutrition process in cell culture with Ayurveda conception.

Nutrition in Ayurveda:

Food is responsible for different aspects of an individual including physical and mental states. A stable healthy diet routinely is essential for healthy body. Indian traditional foods are recognized as functional foods because of the presence of functional components such as body healing chemicals, antioxidants, dietary fibers and probiotics. The body absorbs the nutrients as the result of digestion. Ayurveda states that the food first converts into *rasa* and then followed by successive conversion into blood, muscle, fat, bone marrow, reproductive elements and body fluids^{[6][7]}. Mental health is also essential for a healthy person. Stimulation of mind helps in relaxation through reduction in stress and indirectly improves other body activities^{[8][9]}.

Doshas are classified into *vatta*, *pitta* and *kapha* according to the dominant constituent of the body. Nutrition of body according to the Ayurveda depends on basic principles of Ayurvedic diet are *kedara kulya nyaya*, *khale kapota nyaya*, and *kshira dadhi nyaya*^{[10][11]}. These three

principles describe different aspects of digestion and digested food function as growth and provide nutrients in the body. *Kedara kulya nyaya* is the first principle of Ayurvedic diet. This principle describes the initial digestion process, where one of the basic elements, fire, breaks food materials into biological elements which are circulated throughout the body in the *ahara rasa* via the circulatory system. *Khale kapota nyaya* describes selectivity of nutrient by tissues, where every *dhatu* selects the specific nutrient for its nourishment. For instance, *rasa agni* selects plasma cells, protein molecules are selected by *mansa* cells, while iron molecules are chosen by *rakta agni*. *Kshira dadhi nyaya* governs transformation of *asthayi dhatu* into *sthayi dhatu*. *Rasa asthayi dhatu* transforms into *sthayi dhatu* by the action of *rasa agni*. This principle explains different processes and their concepts involved in growth, development and nourishment of tissues^{[10][11]}.

Concept of Nyayas

1)Ksheeradadhi Nyaya (The Law of Transformation)^[12]:

According to this concept, the preceding dhatus get transformed into the succeeding dhatus, this is on the analogy of transformation of milk into curd. Thus the whole *rasa dhatu* being cooked by the

respective *agni* becomes *rakta*, similarly the *rakta dhatu* changes to *mamsa dhatu* and *mamsa dhatu* changes to *medha dhatu* and likewise and termed as *ksheeradadhi nyaya*. For example, as after the union of *sukra* and *sonita*, when the foetus is developing as a mixture of seven *dhatu*s, the *rasa dhatu* derived from mother is transformed into *rakta* by the *rasadhatwagnipaka*. Through this method of *dhatuparinama*, the foetus develops gradually. From the third month onwards, the foetus is nourished through the *nadi* which connects the umbilicus of the foetus to the heart of the mother. After the establishment of *nadi*, the nourishment of the foetus is conducted by *kedarikulya nyaya*. Even after the delivery, the nourishment of body is by the law of transformation. The *dhatwagnipaka* comprises of both *prasadapaka* and *kittapaka*. In this *nyaya* also, *prasadapaka* produces the nourishing part of the *swadhatu* and the *poshaka bhaga* of the succeeding *dhatu*. And the *malarupa kapha* and *pittas*, which are excreted into *koshtha* form the muconium of the infant which is excreted after delivery.

2) *Kedarikulya Nyaya* (The Law of Transmission)^[13]:

According to this theory, the process of nourishment of tissues can be compared to

the irrigation of different fields by water from a canal. The water first irrigates the nearby fields and only there after the distant ones are irrigated. Thus, the *rasa* after supplying nutrition to *rakta* then, *rakta* proceeds to provide nourishment to *mamsa dhatu*. In this way, the nourishment is taken to all the *dhatu*s by *ahararasa* due to this order each *dhatu* can pick up its own nutrients. According to this concept one *dhatu* alone can get affected without involving other tissues^{5,6}. *Sthayi dhatu*s have already developed and the nourishment of the *dhatu*s is through the *ahararasa*. The nourishment is through the specific *dhatuvaha strotas* and there is a force acting to transport the *ahararasa* to respective sites of the *dhatu*s through the *Strotas*. The stimulation of the muscular *hridaya* for its contraction and relaxation the *vyanavata* is required for the circulation of *ahararasa* in body.

3) *Khalekapota Nyaya* (The Law of Selectivity)^{[14][15]}:

According to this concept, the nourishment of *dhatu*s takes place by selection. The nourishing fluid travels through different channels. Different *dhatu*s get nourished by this nourishing fluid at different stages. Thus, the nourishment of tissues takes through different channels. This is on the analogy of the pigeons carrying grains from

a thrashing field and flying out in different directions. According to this *nyaya* the *ahararasa* goes to various *dhatu*s through different channels, *dhatumargas* and *Strotas* provide them with nutrition. The portion of *ahararasa* meant to provide nourishment to a particular *dhatu* does not come in contact with other *dhatu*s. The portion *ahararasa* meant to nourish *rasa dhatu* passes through its own *strotas* and provides nutrition to it. After providing nutrition to *rasa*, the portion of *ahararasa* meant to provide nutrition to *rakta dhatu* does its job through its specific *strotas* and the goes on till the last *dhatu* gets nourished. *Khalekapota nyaya* clear that the nutrient substances by each one of *sthayi dhatu*s are also specific to each one of them. The nutrients meant for one *dhatu* may not be utilised by another *dhatu*.

4) *Ekkaladhatuposhan Paksha*:^{[15][16]}

This method is proposed by arundatta. Ejected into circulation by *vyanavata ahararasa* enters the seven *strotas* and nourishes the seven *dhatu*s. The site of *rasa dhatu* is *hridaya*, but circulates throughout the body. separate sites have been stated for the seven *dhatu*s, they are present throughout the body. Therefore, the *ahararasa* circulates quickly in the body is stated to nourish all *dhatu*s at the same time. This method of utilisation of the nutrients

derived from the food indicates only nourishment of *sthayi dhatu*s.

Nutrition in in- vitro:

Cell culture is one of major techniques in the life sciences. The basic requirements for cells to proper nutrition are controlled temperature, substrate for cells attachment and appropriate growth medium and incubator that maintains correct pH and osmolality^[17]. A balanced set of common nutrients is required by most cell types. There are many of nutrients required for specific classes of cells. The nutrition of animal cells in tissue culture are amino acids, vitamins, nucleic acid constituents and various accessory growth factors. The optimal concentrations of substances for the maintenance of cell life are required to established and it is a completely artificial feeding solution. The another substance for nutrition are hormones, substrate, growth factors and polypeptide signal molecules. This class of compounds is cells specific and these components required for growth and differentiation of cells.

The most important and crucial step for nourishment of cell culture is selecting appropriate growth medium for the in vitro cultivation^[18]. Cell culture medium is composed of amino acids, vitamins, inorganic salts, glucose and serum. Cell

culture media consist of an appropriate source of energy and compounds which regulate cell survival, proliferation and cellular functions. The medium helps maintain pH, osmolality, regulate individual nutritional requirement and determine individual metabolic characteristics.

Types of Cell Culture Media^[17]

Natural Media - Natural media consist only of naturally occurring biological fluids like plasma, serum, lymph, human placental cord serum, amniotic fluid, tissue extracts like extract of liver, spleen, tumours, leucocytes and bone marrow, extract of bovine embryo and chick embryo clots like coagulants or plasma clots.

Artificial media - Artificial or synthetic media are prepared by adding nutrients (both organic and inorganic), vitamins, salts, O₂ and CO₂ gas phases, serum proteins, carbohydrates, co-factors.

Artificial media are grouped into four categories:

Serum containing media:

Fetal bovine serum is the most common supplement in animal cell culture media. It is used as a low cost supplement. Serum provides carriers for water-insoluble nutrients, hormones and growth factors, protease inhibitors, binds and neutralizes toxic substances.

Serum-free media:

These media are formulated to support the culture of a single cell type. This media called culture media.

Chemically defined media:

These media contain contamination-free ultra-pure inorganic and organic ingredients. It contains pure protein additives, growth factors. Their constituents are produced in bacteria or yeast by genetic engineering with the addition of vitamins, cholesterol, specific amino acids, and fatty acids.

Protein-free media :

Protein-free media do not contain any protein and only contain non-protein constituents. Protein-free media promotes superior cell growth compared to serum contain media.

Basic components of culture media:^{[17][18]}

Culture media contain a mixture of amino acids, glucose, salts, vitamins, and other nutrients. It is available either as a powder or as a liquid form. Each component performs a specific function, as described below:

Buffering systems

It regulates the pH for optimum culture conditions. It also maintained by natural buffering system.

Phenol red

Most of the commercially available culture media include phenol red as a pH indicator. During the cell growth, the medium changes color as pH is changed due to the metabolites released by the cells.

Inorganic salt

Inorganic salts are sodium, potassium, and calcium ions helps to retain the osmotic balance and regulates membrane potential.

Amino Acids

Amino acids are the building blocks of proteins. They are required for the proliferation of cells, stimulates growth and prolongs the viability of the cells.

Carbohydrates

Carbohydrates in the form of sugars are the major source of energy. Most of the media contain glucose, galactose, maltose, fructose.

Proteins and Peptides

Albumin, transferrin, and fibronectin are important in serum-free media. Serum is a rich source of proteins and includes albumin, transferrin, aprotinin, fetuin, and fibronectin. Albumin is the main protein in blood acting to bind water, salts, free fatty acids, hormones, and vitamins, and transport them between tissues and cells. The binding capacity of albumin makes it a suitable remover of toxic substances from the cell culture media. Aprotinin is a protective agent in cell culture systems. Fibronectin is a key player in cell

attachment. Transferrin is an iron transport protein that acts to supply iron to the cell membrane.

Fatty Acids and Lipids

They are important in serum-free media. They are present in serum.

Vitamins

Vitamins are essential for growth and proliferation of cells. Vitamins cannot be synthesized in sufficient quantities by cells and are therefore important supplements required in tissue culture. Serum is the major source of vitamins in cell. The B group vitamins are most commonly added for growth stimulation.

Trace Elements:

Trace elements are supplemented to serum-free media to replace those normally found in serum. They are needed in minute amounts for proper cell growth.

Media Supplements:

Media supplements helps in proliferation and maintain of normal cell metabolism.

Antibiotics:

Antibiotics are used to control the growth of bacterial and fungal contaminants.

Criteria for Selecting Media: ^{[17][18]}

The selection of the media depends on the type of cells to be cultured. Different cell types have highly specific growth requirements so that most suitable media for each cell type must be carefully.

Common cell culture media^[17]

Eagle's Minimum Essential Medium (EMEM), Dulbecco's Modified Eagle's Medium (DMEM), RPMI-1640, Ham's Nutrient Mixtures, DMEM/F12, Iscove's Modified

Discussion:

Ayurveda lays great deal of emphasis upon proper diet for the preservation and promotion of positive health, prevention and cure of diseases. Food taken in proper quantity provides strength, vigor, good complexion and nurtures the health of the tissues. The appropriate quantity of food is responsible for no obstruction in heart, saturation of sense organs, inspiration, expiration and laughing, easy digestion of food, promotion of strength, complexion, growth and development of the body. On the other hand, the proper supplement of nutrients in cell culturing technique is essential for growth and differentiation of cells. Growth in isolated systems provides the opportunity to examine metabolic pathways and nutritional requirements of cells in a controlled manner.

There is nearly cell culture nutrition process equivalent to the Ayurvedic concept of nutrition pathways. Still it can be compared with various metabolic reactions taking place in our body. The anabolic and

catabolic reactions in culture technique can have some resemblance with the Ayurvedic concepts of *prasadapaka* and *kittapaka* respectively. The forethought of our *acharyas* seems evident in giving *dhatuposhana* as well as *malaroop* attributes to the *doshas*, parallel to the concept of anabolic and catabolic changes in modern medicine. As stated there are many of nutrients required for specific classes of cells. Ayurveda also follows that specific substances provides growth and sustenance for specific *dhatu*s. For example, consumption of meat increases *mansa dhatu*. According to Ayurveda, *Khale kapota nyaya* also describes that selectivity of proper nutrients by every *dhatu* helps in differentiation and growth of *dhatu* nourishment.

The different growth medium involves maintain pH, osmolality, regulate individual nutritional requirement and determine individual metabolic characteristics. As stated in *khalekapota nyaya* that nourishing fluid travels through different channels and different *dhatu*s get nourished by nourishing fluid at different stages. Thus, the nourishment of tissues takes through different channels and poses individual characteristics of seven *dhatu*s. The different growth medium uses in for several cell culture techniques. Growth medium works as *ahararasa* for cell

survival, cell proliferation and growth stimulation through different channels. *Khalekapota nyaya* clears that the nutrient substances by each one of *Sthayi dhatus* are also specific to each one of them. The nutrients meant for one *dhatu* may not be utilised by another *dhatu*. As stated in modern cell culture technique that transferrin is a specific growth medium as iron transport protein which acts to supply iron to the cell membrane.

Energy source is an essential nutrient is required for survival, multiplication and differentiation of the cells. But the energy cannot be produced by the cell in adequate quantities. Along with energy, the other essential nutrients are growth factors, generally protein in nature, hormones and other compounds that play crucial roles in cellular processes. The ability to culture cells in chemically defined media provides the potential to determine both qualitative and quantitative requirements of each cell type. The used of defined media in cell culture produces standardization and reproducibility which in turn make transfer of technology among laboratories more straightforward. Growth of cells in culture allows exploration of problems which are not amenable the study in humans. The culturing technique of mammalian cells is an essential for the study of biochemical processes and developmental events. The

development and application of defined media for growth of specific classes of cells provide essential experimental tool to address a wide assay of questions over the complete range of Ayurveda sciences. It explains that meticulous nutrition process that takes part in isolated environment.

But realize on the other side, the major components of media made up of naturally derived products serum. But it then gradually shifted to the use of chemical-based synthetic media. Because naturally made ingredients have their disadvantages. The disadvantage is that batch-to-batch variation. Another drawback is that foreign substances from unidentified sources can contaminate a culture medium. These contaminants include viruses, bacteria, mycoplasma, and endotoxins. The other types of contaminants like plasticizers from plastic instruments or trace elements, even in water. Due to this, it affects the empirical results. These substances also can affect the cells in culture. Some toxic substances are used for sterilization during micro filters process. So that expertise and well equipment are needed.

Cost factor is also another the major issue to affordable everybody in these studies. There is a limitation of expertise, young Ayurveda scholars although they are not clear about their views on the future of Ayurveda. The homeostatic components

are lacking in nourishment of cell culture due to outside environment culturing so that result is not perfect or uncertain. But see as advantages of cell culture technique, it is very useful in investigate normal physiology or biochemical of cells. These are useful in various pathological conditions, virus detection and vaccine development. The government should be prepared a financial development about the issue of cost factor of cell line for the progress and development of Ayurveda. India has to develop some type of policies in the health care system for the further development of Ayurveda. There is a need of research on Ayurveda to refresh and upgrade the tremendous knowledge.

Conclusion:

The epistemology of Ayurveda is based on five basic elements (*mahabhuta*), three dynamic principles similar to humour (*dosha*), seven types of tissues (*dhatu*) and many other unique concepts. Ayurveda is instinctive and holistic, whereas that of the modern biochemistry is based more on experimental, analytical and reductive reasoning. Nutrition of human body is based on absorption of food, assimilation and excretion process. Proper nourishment of body is the need of every living organism. But in cell culture, a cell takes our nutriment outside the internal

environment through different media. A cell artificially nourishes and completes their requirement through natural or synthetic media for growth and differentiation. This review and exploration is carried out that the methods of cell culture nutrition and evidence of cell line approach of biomedical science is need of Ayurveda. The Ayurveda sector should urgently recognize and address the need for scientific evidence, appropriate organization and harsh research in accordance with good expertise to move towards evidence-based Ayurveda. Ayurveda has a top down, holistic understanding of organisms and their interactions with the environment at a functional level and not so much at the molecular level. Biochemistry science as based more on the rationalism, reductionism with deeper understanding of molecules, cells, organs or diseases. Ayurveda principles are largely holistic and based on an epistemology that is very different from modern bioscience which is more reductionist in its approach. The methods and instrument used in biochemistry are designed to understand genes, atoms, molecules, cells, tissues and organisms. Ayurveda needs the focus on systemic and functional aspects of cells, qualitative analysis of drugs. The current day system of Ayurveda approach aims to

be more holistic but it also derived from a systematic synthesis of data obtained from the reduced view of system, using extensive computational and mathematical modelling of inter-linkages.

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