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Factors affecting in cell and tissue culture for cell and tissue growth and development

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Abstract
Cell or tissue culture relies on the fact that many cells in plant, animal and human have the ability to regenerate a whole organ of plant, animal or human. Single cells, plant cells without cell walls pieces of leaves, or root cell, embryonic cell, muscle tissue can often be used to generate a new organ, tissue on culture media giving the required growth factors like nutrients and hormones. Significant growth factors like moisture, light, oxygen, temperature, nutrition, culture media, hormones and growth regulators, sources of sample, sterile environment, incubator, cell density in 3D culture and microfluidic factors during cell or tissue culture method were noted well.

Keywords: cell and tissue culture, growth factor, contamination, micropropagation

1. Introduction

Growth factors are an important component for cell and tissue culture which includes cell physiology that is meant the biological study of the cell's mechanism and interaction in its environment. The term physiology refers to all the normal activities that take place in a living organism in the cell and tissue culture method. This includes, human cell, animal cells, plant cells and microorganisms. All of these activities may contaminate in the cell culture steps which is regarded as nutrition, environmental response, cell growth, cell division, reproduction and differentiation (Hossain *et al*, 2012, Hammad and Rosna, 2008, Hossain and M. Uddin, 2018) [3].

Hossain *et al.* (2014) stated that water absorption by root, production of food in the leaves, and growth of shoots towards light are examples of plant physiology. Experimental approach in cell physiology is an important aspect in cell physiology because it utilizes the growth factors properly in the experimental methods in order to solve any scientific issue related to physiology in culture.

Cell culture is the complex process by which cells are grown under controlled conditions, generally outside of their natural environment (Hossain *et al*. 2014). In practice, the term "cell culture" has come to refer to the culturing of cells derived from multi-cellular eukaryotes, especially human, animal and plant cells. However, there are also cultures of fungi and microbes, including viruses, bacteria and protists (Hammad and Rosna, 2019).

Alvarado and Tsonis (2006) stated that during the cell developmental process, genes are activated that serve to modify the properties of cell as they differentiate into different tissues. Development and regeneration involves the coordination and organization of populations cells into a blastema, which is a mound of stem cells from which regeneration begins. Kumar *et al*. (2007) explained that the differentiation of cells means that they lose their tissue-specific characteristics as tissues remodel during the regeneration process.

Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 37 °C, 5% CO_2 for mammalian cells) in a cell incubator. Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes. Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the cell growth medium. Recipes for growth media can vary in pH, glucose concentration, growth factors, and the presence of other nutrients (Jill, 2015).

Cross-contamination can be a problem for scientists working with cultured cells lines (Jill, 2015). Studies suggest anywhere from 15–20% of the time, cells used in experiments have been misidentified or contaminated with another cell line. Problems with cell line cross-contamination have even been detected in lines from the NCI-60 panel, which are used routinely for drug-screening studies (Drexeler *et al*. 1999; Drexeler *et al*, 2001). Major cell line repositories, including the American Type Culture Colection (ATCC), the European Collection of Cell Cultures (ECACC) and the German Collection of Microorganisms and Cell Cultures (DSMZ), have received cell line submissions from researchers that were misidentified by them (Cabrera, 2006, Chatterjee, 2007; Liscovith and Ravid, 2007).

Some cells naturally live in suspension, without being attached to a surface, such as cells that exist in the bloodstream. There are also cell lines that have been modified to be able to survive in suspension cultures so they can be grown to a higher density than adherent conditions would allow. Adherent cells require a surface, such as tissue culture plastic or micro-carrier, which may be coated with extracellular matrix components to increase adhesion properties and provide other signals needed for growth and differentiation (CC, 2006).

1.1.1 Moisture

Cell growths are restricted in moisture supply in the media and improves nutrient uptake. Normally 65% of moisture cells are died in the cell or tissue culture method (Hossian *et al*. 2018).

1.1.2. Temperature *in vitro*

When it comes to temperature, it would not be separated from the process of photosynthesis, respiration, and evaporation in plants. Temperature affects the water content in plants that is one thing that is important in the growth and development. Temperature also affects the performance of enzymes that exist in plants. Usually 25 ^{0}C in the laboratory (Hossain *et al*. 2014).

1.1.3. Light *in vitro*

UV light iddivided in to three. UV-C wave length from 100nm to 280nm. UV-B 280nm to 315nm and UV-A 315nm to 400nm. The wave length of 254nm (UV-C) is giving maximum germicidal activity and it is not too irritating. Sun light affects green plants because sunlight determines the photosynthesis process of plants. Photosynthesis is the fundamental process in plants to produce energy in the growth and development. Besides, in the process of photosynthesis, sunlight also affect plant growth physically (Hammad and Rosna, 2008, Hossain *et al*, 2012) [3].

1.1.4. Oxygen *in vitro*

Oxygen is a limiting factor in any organism. These conditions make the organisms need oxygen. Parts of plant roots require good aeration to get oxygen. Good aeration to improve root respiration process to circulate nutrients in the soil to the leaves. The only cells that do survive in an environment with 20% oxygen are lung alveolar cells, and shortly after blood is oxygenated the oxygen level in arterial blood falls to 10.5-13% (80-100 mm Hg). Even more striking is that many organs function normally at oxygen levels ranging from 2-8% (19-70 mm Hg) (Hossain *et al*, 2012) [3]

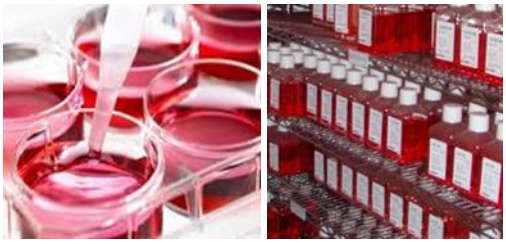
1.1.5. Nutrition *in vitro*

Plants need nutrients for its survival. Nutrients needed in large quantities such as carbon, oxygen, hydrogen, nitrogen, sulfur, potassium, calcium, phosphorus and magnesium. These elements are referred to as the mean macronutrient nutrients needed by plants in large numbers. Nutrients needed for plants not deficient, that is to grow and develop imperfectly (Hossain *et al* 2015).

1.1.6. Culture Media and plant growth regulator/hormone *in vitro*

The growth of cultures takes place in the specific culture media. In general, these media should have a composition that provides the necessary nutrients and growth factors in the right proportion. Culture media consists of certain major inorganic nutrients such as nitrogen, phosphorus, potassium, calcium, magnesium and sulphur. Plant growth regulators such as auxins, gibberellin and cytokinins should be present in the right proportion for root and shoot formation. A growth factor is a naturally occurring substance capable of stimulating cellular growth (Thomas and Abbot, 2007) [5] proliferation, healing, and cellular differentiation. Usually it is a protein or a steroid hormone. Growth factors are important for regulating a variety of cellular processes. Growth factors typically act as signaling molecules between cells. Examples are cytokines and hormones that bind to specific receptors on the surface of their target cells (Hossain and Uddin, 2018).

Plant tissue culture



Animal or human cell culture

Fig 1: Explants or cell in media (www.sites.psu.edu, www.biocompare.com), [www. innoprof.com]

1.1.7. Source of cell of plant, animal and human *in vitro*

Several plant parts can be used as explants to initiate a plant tissue culture. However, each plant organ differs in its rate of growth and regeneration because the cells in that organ exist in a particular developmental stage. Organs also differ in their metabolic activity and capacity to transport and utilize growth regulators. Generally, meristematic tissues such as the root tip, stem tip and auxiliary bud tip are good explants because they show the most rapid rate of cell division. For for animal and human sources like skin, liver, bone marrow, blood, eyes, brain, muscle etc. (Hammad and Rosna, 2008, Hossain *et al*, 2012) [3].

1.1.8. Sterile Environment *in vitro*

Under sterile conditions, plant, animal and human organ or tissue cannot be contaminated during culture, often to produce clones of a plant, animal and human tissue (Agricare, 2005). The medium used for growing plant tissue cultures provides conditions that are conductive for the growth of microorganisms. It is, therefore, very important to retain the sterility of the medium. Autoclaving of culture media before use, transferring tissues into culture media under a Laminar Air Flow cabinet, and following aseptic techniques should be strictly enforced for a successful tissue culture. The water used for preparing the medium should be rendered sterile through distillation or reverse osmosis (Hossain and Uddin, 2018).

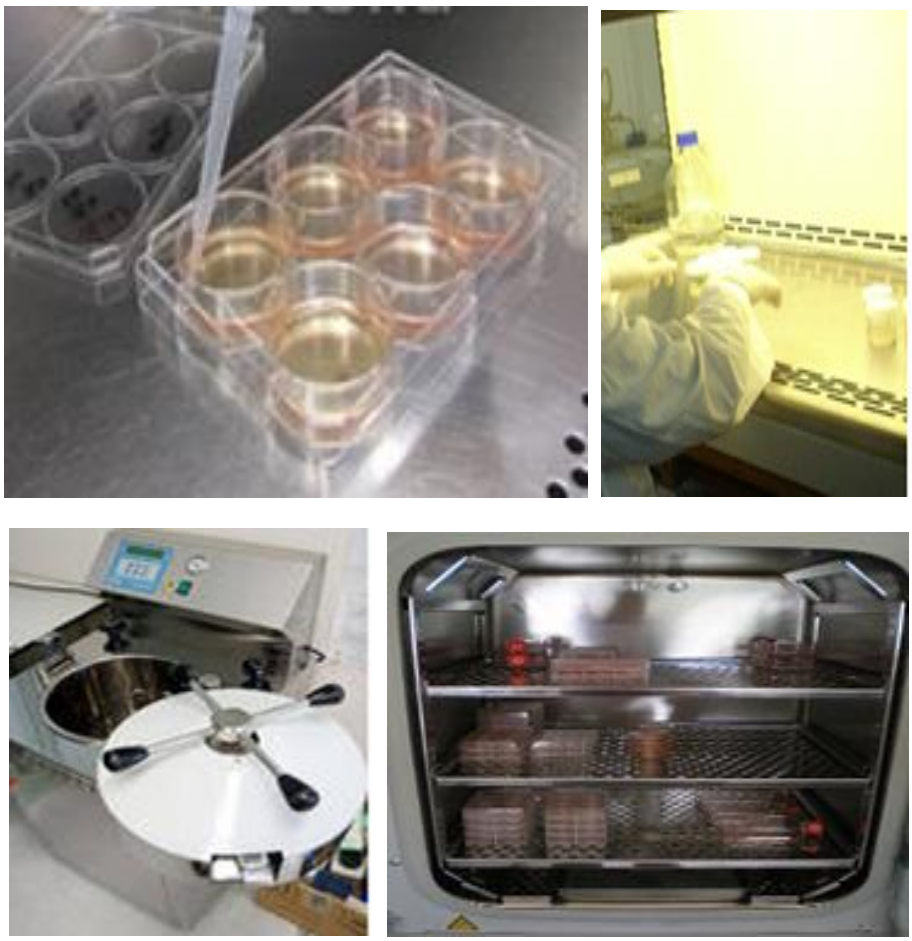


Fig 2: Sample preparation in laminar flow and autoclave and growth chamber (www.upload.wikimedia.org)

1.1.9 Incubator

An incubator is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as the carbon dioxide (CO_2) and oxygen content of the atmosphere inside. Incubators are essential for a lot of experimental work in cell biology, microbiology and molecular biology and are used to culture both bacterial as well as eukaryotic cells (Hossain and Uddin, 2018).

1.1.10 High cell-density

The fed-batch strategy is typically used in bio-industrial processes to reach a high cell density in the bioreactor. Most of the feed solution is highly concentrated to avoid dilution of the bioreactor. Production of heterologous proteinusing fed-batch cultures of recombinant microorganisms has been studied. The nutrient directly affects the growth rate of the culture and helps to avoid overflow metabolism (formation of side metabolites) such as acetate for *Escherichia coli*, lactic acid in mammalian cell cultures, Ethanol in yeast oxygen limitation (anaerobiosis) (Hossain and Uddin, 2018).

2. Factors affecting 3D cell culture

A 3D cell culture is an artificially-created environment in which biological cells are permitted to grow or interact with their surroundings in all three dimensions. This is an improvement over the previous method of growing cells in 2D (on a petri dish) because the 3D model more accurately models the *in vivo* cells. These three-dimensional cultures are usually grown in bioreactors, small capsules in which the cells can grow into spheroids, or 3D cell colonies. Approximately 300 spheroids are usually cultured per bioreactor (Stephen 2013) [6].

2.1 Properties of 3D culture cell

In living tissue, cells exist in 3D microenvironments with intricate cell to cell and cell to matrix interactions and complex transport dynamics for nutrients and cells. Standard 2D, or monolayer, cell cultures are inadequate representations of this environment, which often makes them unreliable predictors of *in vivo* drug efficacy and toxicity. 3D spheroids more closely resemble *in vivo* tissue in terms of cellular communication and the development of extracellular matrices. These matrices help the cells to be able to move within their spheroid similar to the way cells would move in living tissue. Thus the spheroids are improved models for cell migration, differentiation, survival, and growth. Furthermore, 3D cell cultures provide more accurate depiction of cell polarization, since in 2D, the cells can only be partially polarized. Moreover, cells grown in 3D exhibited different gene expression than those grown in 2D (Stephen 2013) [6].

The 3D (third dimension) of cell growth provides more contact space for mechanical inputs and for cell adhesion, which is necessary for integrin ligation, cell contraction and even intracellular signaling. Normal solute diffusion and binding to

effector proteins (like growth factors and enzymes) is also reliant on the 3D cellular matrix, so it is critical for the establishment of tissue scale solute concentration gradients *(Francesco, 2007).*

2.2.3 D culture methods

In general, there are two types of 3D culture method: Scaffold technique: Scaffold techniques include the use of hydrogels and other materials. Scaffold-free technique: Scaffold-free techniques employ another approach independent from the use scaffold. For example: the use of low adhesion plate and micropatterned surfaces (Vivien, 2013) [7].

2.3. Factors

2.3.1 Microfluidics

The various cell structures in the human body must be vascularized to receive the nutrients and gas exchange help that they need to survive. Similarly, 3D cell cultures *in vitro* require certain levels of fluid circulation, which can be problematic for dense, 3D cultures where cells may not all have adequate exposure to nutrients. This is particularly important in hepatocyte cultures because the liver is a highly vascularized organ. The study cultured hepatocytes and vascular cells together on a collagen gel scaffold between microfluidic channels, and compared growth of cells in static and flowing environments, and showed the need for models with tissues and a microvascular network (Vivien, 2013) [7].

2.3.2 Pharmacokinetic and pharmacodynamic effects

A primary purpose of growing 3D cell spheroids *in vitro* is to test pharmacokinetic and pharmacodynamic effects of drugs in preclinical trials [9]. Toxicology studies have shown 3D cell cultures to be nearly on par with *in vivo* studies for the purposes of testing toxicity of drug compounds. When comparing LD50 values for 6 common drugs: acetaminophen, amiodarone, diclofenac, metformin, phenformin, and valproic acid, the 3D spheroid values correlated directly with those from *in vivo* studies. Although 2D cell cultures have previously been used to test for toxicity along with *in vivo* studies, the 3D spheroids are better at testing chronic exposure toxicity because of their longer life spans. The matrix in 3D Spheroids causes cells to maintain actin filaments and is more relevant physiologically in cytoskeletal organization and cell polarity and shape of human cells. The three-dimensional arrangement allows the cultures to provide a model that more accurately resembles human tissue *in vivo* without utilizing animal test subjects (Glauco, 2010) [8].

Drug interaction, such as dose dependent cell viability, cell-cell/cell-matrix interaction, and/or cell migration, but the available assays are not optimized for 3D cell culturing. The next challenge faced by 3D cell culturing is the limited amount of data/publications that address mechanisms of drug interaction, cell differentiation, and cell-signalling in *in vitro* 3D environments and correlate results with *in vivo* drug response. (www.Nature.com)

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