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FUNDAMENTALS OF CELL CULTURE

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The purpose of this review is to provide a fundamental understanding of cellular technologies for students, doctors, masters.

Cell culture is an important technique in both cellular and molecular biology given that it provides the best platform for studying the normal physiology and biochemistry of cells.

The reason for focusing on cell culture systems is because they are indispensable tools for basic research and a wide range of clinical in vitro studies. The review provides a critical analysis: both positive and negative aspects of cell technologies.

Cell cultures can be used to mimic living conditions. The disadvantages include the fact that cell culture is still not an absolute model of a living organism, but only resembles the living conditions of living organisms. A further disadvantage is with contaminants of cell culture; these can be divided into chemical and biological contaminants.

Working with cell culture is a financially costly laborious process: it requires modern equipment, specialized laboratory and well trained staff.

The article discusses in detail the issues of the use of cell cultures in medicine, pharmaceutical industry and biology.

Key words: cells, living, conditions, cell culture, laboratory technique

Cell culture is the laboratory technique of growing and maintaining the cells of multicellular organisms (plants and animal cells inclusive) in a favourable artificial environment conducive for growth. It is the maintenance of animal cells (inclusive of plant and human cell lines) in vitro. Such an artificial environment or growth medium mimics the internal environment of the organism from which the cell was obtained from; and they support the propagation of the cells to be cultured in vitro. Cell culture is also the removal of cells from an organism (i.e. from their parent tissue) and their subsequent growth in vitro under special controlled growth conditions. This process of removing cells or tissues from their normal in vivo environment (i.e. from their parental host organism) and maintaining their growth in vitro in an artificial growth environment is generally known as explantation. Such cells or tissues are said to be explanted since they now survive outside their host organism. An explant is simply defined as a fragment of tissue that is transplanted from its original (parental) host organism and maintained in an artificial growth medium in vitro [4].The survival or viability of cells or tissues outside of their host or parental organism after explantation is critical because once a cell or tissue is explanted from its normal physiological in vivo environment, maintaining its optimal growth in vitro is fundamental to the success of the experimentation. Cell culture technique is also used for the study and cultivation of viruses, protozoa and other obligate intracellular parasites such as Chlamydia and Rickettsia. To ensure viability of the explanted cells or tissues, the in vitro artificial growth environment must be inundated and fashioned with all necessary requirements that mimic the normal in vivo environment from which the cells/tissues was obtained from. There are several environmental factors that must be provided for the successful in vitro cultivation of living cells and tissues outside their physiological in vivo environment. Culture is generally the microbiological laboratory technique in which the growth of microorganisms in a growth medium (solid, liquid or broth) is enhanced for visibility and easy study. Such a medium (which is usually placed in a Petri dish) contains nutritive substances/materials that support the growth of the organisms under certain conditions [5]. For example, bacteria grow best at 37° C for 18-24 hrs while fungi grow best at 28° C for 18-24 hrs. It is noteworthy that the medium for the growth of a bacterium and fungus varies, and so does the temperature at which they grow.

Though cells that can be grown through cell culture can be of prokaryotic or eukaryotic origin, in research the term "cell culture" refers mainly to the culturing of cells that are either of human, microbial, animal or plant origin. The culturing of fungal or bacterial cells must not be mistaken for cell culture. But for the purpose of this topic, the term cell culture and tissue culture shall be used interchangeably. Cells derived from ei-

ther animals, humans or plants will continue to grow if supplied with the correct nutrient and environmental conditions necessary for growth to take place [1]. The cultured cells are capable of dividing and increasing in size until their growth is limited by some environmental conditions such as the depletion of growth nutrients. Cell culture encompasses organ culture and other in vitro culture techniques in which cells derived from their parent tissues (as dispersed cells) or from particular cell lines/strains and from primary cell cultures are cultivated in vitro in specialized growth medium that mimic the natural environment from which the cells were initially derived from. Cell culture techniques have several applications. It is applied in tissue engineering, toxicological research, immunological research, proteomics, pharmacology (especially in drug design), and in other advanced molecular biology manipulations [8].The materials and/or information provided present written text, are only an elucidation of the basic principles and knowledge that guides cell or tissue culture techniques. These dos and don'ts of cell culture techniques have been succinctly highlighted in this section of this review for the purpose of tutelage and research. The ensuing sections of this text have concisely elucidated these basic principles or fundamentals of tissue culture in such a way that the reader will be fully knowledgeable about cell culture techniques [6].

Historical development. Cell culture as a basic molecular science has been in existence for over 10 decades now. Its development even till date has been spurred by the innovative works of scientists in time past. Sydney Ringer in the early 19th century developed a salt solution called Ringers solution which contains sodium, potassium, calcium and magnesium chlorides. This salt solution was used to maintain the heart of an animal outside of its body. Wilhelm Roux, a German zoologist showed in 1885 that the neural plate from chicken embryos could be removed and maintained in warm saline solution for several days outside the chicken's embryo. Cell culture was successfully undertaken by the work of the American born scientist, Ross Granville Harrison who in 1907 showed that the nerve fibres of frog/ amphibians can be removed and maintained outside the cell in conditions that can favour its proliferation. In 1913, the French medical scientist, Alexis Carrel successfully showed that the heart of a chicken embryo could be removed and kept for a period much longer than the normal lifespan of a chicken. These achievements set the foundation for the development of cell culture which advanced greatly in the 1940s and 1950s. The fields of virology was also driven further in the area of vaccine development as a result of cell culture techniques which allowed the culturing of viruses for the production of vaccines. Earle and his colleagues in 1943 developed mouse lymphocyte cell lines. In 1949, John Franklin Enders and colleagues successfully showed that viruses can be grown in cell culture. They used monkey kidney for this purpose and was awarded the Nobel Prize for the discovery of a method for growing the virus in monkey kidney cell cultures, a technique which was used for the mass production of the Salk polio vaccine. In 1952 the HeLa cell lines was developed by George Otto Gey and colleagues. Harry Eagle in 1955 developed a defined cell culture media for cell culture techniques. Their works developed a well-defined nutrient mixture containing amino acids, vitamins, carbohydrates, salts, and serum which is used even till date. This replaced the tissue extracts previously used to grow cells. This medium helped to maintain pure cell lines indefinitely. It gave impetus to the studying of the cellular processes specific to particular organs or tissues of organisms with greater simplicity. Kleinsmith and Pierce discovered the pluripotential stem cells in 1964, and in 1970 the laminar flow cabinets for cell culture was developed. The laminar flow cabinets are very important in cell culture laboratory because they provide a sterile environment for cell culture techniques to take place [13]. Innovations and discoveries continued in the field of cell culture until the early 1970s when the hybridoma cell lines was developed. The development of hybridoma cell lines was made possible by the works of Cesar Milstein, Georges Köhler and Niels Kaj Jerne. This earned them the Nobel Prize in Medicine and Physiology in 1984. Hybridoma cell lines are immortalized cell lines formed by the fusion of a normal cell with an immortal (tumour) cell. They are used for the production of monoclonal antibodies. Monoclonal antibodies are antibodies produced from a single clone of B cells. Another groundbreaking discovery and development in cell culture occurred in 1998 when James Thomson and John Gearhart independently showed the first culturing of human embryonic stem cells in culture [15]. This brief history on cell culture has incompletely enumerated some of the important discoveries in this field. Nevertheless, there are plethora of advances and discoveries in the field of cell culture especially in the area of stem cell research (which is gradually growing) [10] which are spurring the medical and biomedical sciences into a greater height.

Factors affecting cell culture success. Cell or tissue culture experimentations should not be carried out in the regular laboratory space where other laboratory investigations are undertaken. This is critical to avoid contamination of cells in the cell culture plates and also to ensure that all the physiochemical environmental factors that encourage optimal growth of the cells are provided [3]. Thus, cell culture experimentations should be carried out in a specialized laboratory or an area in the regular laboratory that is secluded from the usual laboratory area in order to achieve optimal result. Some key environmental conditions (i. e. the physiochemical or physicochemical environmental growth factors) must be met in order to achieve optimum cell/ tissue culture technique. Some of these environmental conditions that must be met for cell culture to take place smoothly are highlighted in this section.

Figure 1 – Dulbecco's Modified Eagle Medium (DMEM) for cell culture experimentations (source: the manufacturer)

The microenvironment in which cell culture technique is basically carried out is unique and quite different from the traditional microbial cultures that also occur in vitro in the sense that tissue culture support the growth of "living cells or cultures" derived from their parent cells. Such cells are manipulated and grown in such a way that they mimic the actual natural environment of the host organism from which these cells or tissues where extracted from. Therefore, it is critical that certain physiochemical and environmental factors including temperature, oxygen, pressure and CO_2 of the growth medium (i.e. the cell culture growth medium) is performing at optimal levels that allow these cells to be manipulated in vitro. Controlling these physiochemical environmental factors (even though they may not always be defined in most cell culture techniques) at an equilibrium state is vital to the unperturbed growth of the cells. The growth nutrients for the cells in the culture also provide some supplements such as serum that contribute to the optimal growth of the cells or tissue in the culture [7].

Cell counting is typically undertaken using a haemocytometer. The haemocytometer counts cells manually under the microscope. There are other automatic cells counting machines such as the countess machine which can be used to count cells in the cell culture lab. Cell growth can also be measured by using spectrophotometer. Spectrophotometer is a device that measures the optical density of cells in suspension. The results of a spectrophotometer are extrapolated in order to determine the growth of the cells. A haemocytometer has an indention down the centre where cells can be suspended in a liquid, and then the numbers of cells in a particular sized square are counted to calculate the overall cell count.

Classification of cell culture. In the cell culture laboratory, there are many cell culture techniques that are routinely engaged or carried out. These various types of cell culture techniques are highlighted in this section [12]. Primary cell culture are cells obtained directly from an organism and are directly plated in a cell culture dish or flask. They comprise cells of a tissue or organ obtained from an organism and immediately transferred to a suitable cell culture environment conducive for growth. Such cells will attach to the medium, divide and grow exponentially. They are generally termed primary cell cultures. Primary cell cultures have a limited life span. They will only last for a short period of time (usually days to weeks). Their only advantage is that they may exhibit some physiological behaviour similar to that obtainable in vivo because they are freshly isolated cells. Primary cell cultures are usually unstable and require some time to adapt to the in vitro environment they are in-

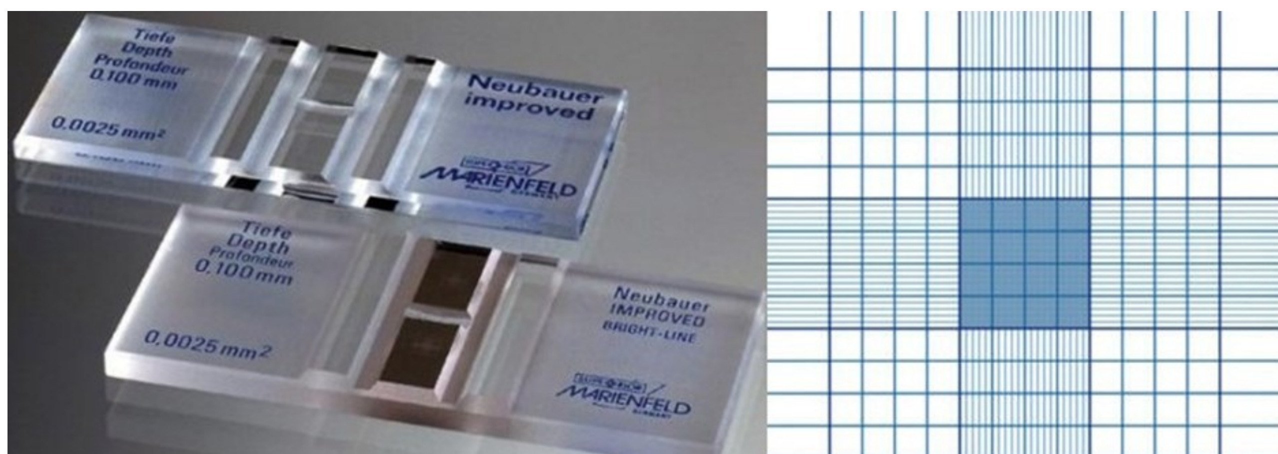

Figure 2 – Illustration of haemocytometer with the squared area (arrow) for counting particular cell types (source: Creative Commons Library)

troduced to. In addition, some cells in primary cell culture may sustain injury during their isolation and preparation, and thus eventually die in the process. In primary cell cultures, a series of enzymatic and mechanical disruptions of the tissues or organs and selection steps are usually employed to isolate the cells of interest from a heterogeneous population of cells. Some examples of primary cell cultures or cell lines includes: macrophages, natural killer (NK) cells, B and T cells, dendritic cells and cells of the spleen (splenocytes). These cells are all cells of the immune system. They are used in primary cell cultures to decode the effects of some certain substances (e.g. drugs) on the unctions and proliferation of cells of the immune system[17]. Secondary cell culture These are cells taken from a primary cell culture and are passaged (or subcultured) into a new and fresh cell culture flask/disk containing new growth medium. Passaging which can also be referred to as sub-culturing is the transplantation of cells from one cell culture vessel to another. Passaging gives cells the chance to expand and increase in population. A higher cell growth is usually achieved due to the addition of fresh growth medium and the introduction of other environmental conditions. Normally, the number of cells obtained from a primary cell culture are may not be enough to create sufficient cells required for a graft, and this warrant the need for Passaging of cells obtainable in secondary cell culture. Secondary cell cultures are transformed and immortalized cell lines with infinite growth and proliferation capacity. They are usually derived from human carcinomas/tumours. Such cells have been transformed in the sense that they have lost sensitivity to factors associated with growth control and thus can grow unlimited. Secondary cell cultures are more easily cultured than the primary cell cultures. Some sources of secondary cell cultures include: embryos and tumours or transformed cells such as HeLa cells and Chinese hamster ovary (CHO). Secondary cell culture has applications in a range of areas such as in vaccine production and drug screening.

Suspension cell cultures These are cells that grow freely and unattached to any surface. Such cells are cultured in suspensions of growth medium. They are maintained in a cell culture flask without any adherence to any surface. Examples of cells cultured in suspension include the cells of the blood such as hematopoietic cells. Such cells are engineered to grow in suspensions. They grow in a very much higher proportion. Adherent cell cultures These are cells that attach or adhere to the surfaces of the cell culture flask used for their culturing. They are referred to as anchorage-dependent cells. These cells are cultivated in suitable growth medium that is especially suited and treated to allow adhesion and the spreading of the cells. The cell culture flask used for adherent cells are usually coated with materials that increase their adherence features and provide signals needed for their growth and proliferation in the cell culture medium.

Cell lines. A cell line is a cell that has undergone mutation and series of genetic manipulations, and will not undergo apoptosis after a limited number of passages (sub-culturing). Apoptosis is defined as programmed cell death. It is a cell death that occurs by a biologically-controlled intracellular process that involves the fragmentation and cleavage of the host cells nucleic acid (particularly the DNA). When cells from the first culture (usually taken from the organism) are used to make subsequent cultures, a cell line

Figure 3 – Water bath containing cell culture flasks (source: Creative Commons Library)

is established. Immortal cell lines can replicate indefinitely due to manipulations of their genetic material and the maintenance and sustenance of optimum nutrient and environmental conditions. Cell lines are cells or cell cultures obtained after the first subculture of a primary cell culture. This primary cell culture becomes known as a cell line or sub-clone after the first subculture has taken place. Cell lines derived from primary cultures usually have a limited life span. But as these cells are passaged, those cells with the highest growth capacity predominate. This will result in a degree of genotypic and phenotypic uniformity of the population of cells that will be produced. Such cells produced in this way are generally referred to as cell lines. Cell lines are obtained or sourced for research purposes in any of the following ways:

- Through primary culture
- Through Passaging or sub-culturing
- Through buying and borrowing either from already established cell collection centers such as the American Type Culture Collection (ATCC). Cell lines can also be sourced from research laboratories and institute in possession of cell lines. Cell lines obtained by borrowing are usually not too good because they can be contaminated by bacteria or mycoplasmas

Figure 4 – Cell culture work (source: Creative Commons Library)

Why is cell, tissue or organ culture embarked upon despite the ethical issues surrounding its development, acceptance and usage? Cell culture has application in a variety of biomedical sciences and even in the industry. It has become one of the major tools used in the life sciences world over. Its application has greatly revolutionized the field of medicine, biotechnology and even agriculture owing to the many useful products derived from it. Cells are cultured for so many reasons. Some of these reasons are highlighted below.

- Cell culture is an important source of pharmaceuticals such as vaccines, proteins, antibodies, hormones and even the anti-diabetic hormone called insulin.
- It helps to study the metabolism of living cells including cells of microbes, plants and animals.
- Cell culture helps to provide evidence on the function and development of specific cells.
- It is used for the isolation and growth of cancerous cells for further studies.
- Cell culture gives a clue as to how cancerous cells develop and spread.
- It can be used for the screening of putative drugs in the pharmaceutical industry during novel drug development.
- It is a tool for investigating the numerous processes relating to human health and diseases.

Cell culture is used to test new products including drugs, cosmetics, vaccines and other pharmaceuticals for toxicity. Cytotoxicity is the cellular damage of metabolic pathways, structures and intracellular processes of a living organism which ultimately result in the loss of function or impaired metabolic function [16]. In some cases, cytotoxicity may lead to the loss of viability of the lining cells or tissues. Most in vitro experimentations (particularly cell culture techniques) are mainly aimed at determining the probable toxicity or cytotoxicity of substances (e.g. drugs, cosmetics and vaccines) that is being tested using cell culture techniques. And because these tested materials or substances are used in vivo by living organisms (inclusive of humans) for treatment and other beneficial purposes, it is critical that they are certified safe to the host's body and thus portend no danger or toxicity when used. Cytotoxicity testing is used to determine the level of toxicity of a substance at the cellular level either in vivo or in vitro. It can be carried out in vivo (i.e. in laboratory animals) or in vitro (as is applicable with cell culture). Cytotoxicity testing is crucial for the testing of products such as drugs and vaccines as aforementioned before they are released into the market for public consumption [9].

Main applications of cell culture work. Cell culture technique is defined as the process by which prokaryotic, eukaryotic or plant cells are grown or cultured in vitro under controlled laboratory conditions. Cell culture techniques have applications in the following fields (14):

Disease diagnosis. Cell culture techniques are applied in clinical medicine for the diagnosis of infectious diseases especially diseases caused by pathogenic viruses. Cell culture techniques aid in rapid viral detection from clinical samples. It also aids in the early treatment of viral infections once the causative viral agent have been detected. Over the years, viral disease diagnosis has traditionally relied on the isolation of viral pathogens in cell cultures which some perceive as being slow and requires special technical expertise. However, advances in cell culture-based viral diagnostic products and techniques including but not limited to cryopreserved cell cultures, centrifugation-enhanced inoculation, precytopathogenic effect detection, co-cultivated cell cultures, and transgenic cell lines have made cell culture to be useful for the diagnosis of viral diseases.

Biomedical research. In biomedical research, cell culture techniques are most preferable than the use of animals for research. Since the use of animals such as monkeys and chimpanzees for research could lead to the extinction of these animals, cell culture techniques is a good alternative and replacement to prevent the extinction of some wildlife. Cell culture techniques can be applied in biomedical research especially in the area of studying some molecular disease processes, and finding out ways via which these diseases of non-microbial origin could be better treated. With the application of cell culture techniques in biomedical research, improved and prompt ways of detecting disease causative agents could be developed. Cell culture techniques could also be used as model system to *study* basic cell biology, metabolism and the physiology of living systems.

Virology. In the field of virology, animal cell culture techniques can be used to replicate the viruses used for vaccine production instead of using animals for this purpose. Cell culture techniques can also be used to detect and isolate pathogenic viruses from clinical samples. It can also be used to study the growth and develop-

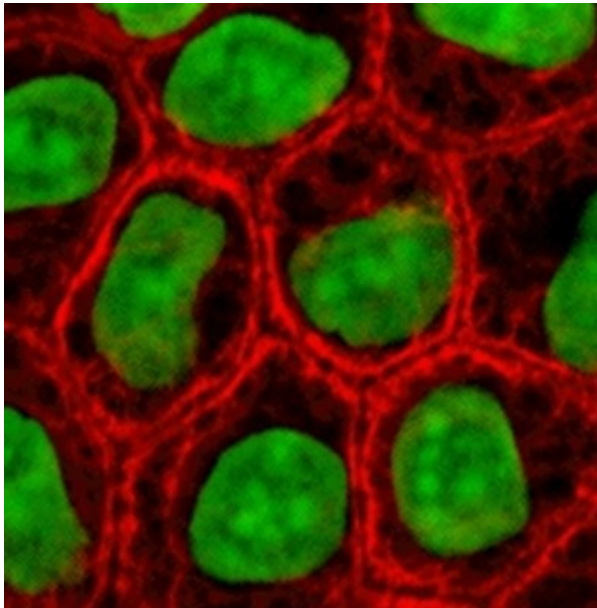

Figure 5 – Stained epithelial cells visualized by scanning laser confocal microscopy. The image shows how keratin cytoskeletal filaments are concentrated around the edge of the cells and merge into the desmoplakin which is located at (source: Creative Commons Library).

ment cycle of viruses. Cell culture techniques can also be used in virology to study the mode of infection of viral disease agents.

Genetic engineering. In genetic engineering, cultured animal cells can be used to introduce new genetic material like DNA or RNA into another cell. Such exchange of genetic information amongst cells or organisms can be used to study the expression of new genes and its effect on the health of the recipient host cell [2]. The recipient host cell starts expressing novel proteins that could be of immense industrial and medical importance. Animal cell cultures are used to produce commercially important genetically engineered proteins or immunobiologicals such as monoclonal antibodies, polyclonal antibodies, insulin, anticancer agents and hormones.

Model systems. Cell culture techniques are used in model systems to study the effect of drugs in human or animal host. It can also be used to study the process of aging in humans. In model systems, cell culture techniques are used to study the major triggers for ageing in man. It can also be used to study how host cell and disease causing agents like bacteria, fungi and viruses interact in vivo.

Cancer research. Cell culture techniques is used in cancer research to study the basic difference between normal cells and cancer cells since both cells can be cultured in vitro in the laboratory. Normal cells can be converted into cancer cells by using radiation, chemicals and viruses. This allows the mechanism and cause of cancer to be studied in vitro using cell culture techniques. Cell culture techniques can also be used to determine the effective chemotherapeutic drugs that can selectively destroy only cancer cells without harming the host cells since most cancer drugs have several untoward effects on the host [11].

Toxicity testing of novel drugs. Cell culture techniques can be used to study the effects of novel drugs, cosmetics and other chemical agents in order to determine not just their efficacy but also the level of their toxicity (i.e. cytotoxicity). The toxicity of the newly developed drugs to vital organs of the body such as the liver and kidney (that are involved in drug metabolism) is also evaluated using cell culture techniques. Drug dosages for novel drugs can also be determined using cell culture techniques.

Gene therapy. *Gene therapy* is an experimental technique that uses *genes* to treat or prevent disease especially molecular or non-infectious diseases such as cancer. It allows clinicians to treat a genetic disorder by inserting a functional *gene (to replace a dysfunctional gene)* into a patient's cells instead of using the conventional treatment methods such as the use of drugs, chemotherapy or surgery. In gene therapy techniques, a dysfunctional gene is replaced with a functional gene. Through cell culture techniques, cultured animal cells are genetically altered and made functional so that they can be used in gene therapy techniques. Briefly, cells are removed from the patient lacking a functional gene or missing a functional gene; and the extracted cells are cultured in vitro through cell culture techniques. These dysfunctional genes are replaced by functional genes. Gene therapy uses a vector, typically a virus, to deliver a gene to the cells where it is needed. Once inside the host cell, the host cell's gene-reading machinery uses the information in the introduced functional gene to build ribonucleic acid (RNA) and protein molecules which will now replace the lost activities of the replaced dysfunctional gene.

Vaccine development. Cell culture techniques can be used in vaccine development since they help to culture animal cells in vitro. Cultured animal cells are in turn used in the production or propagation of viruses that are used to produce vaccines. These vaccines are used clinically for the prevention of communicable diseases caused by pathogenic viruses including measles, polio, rabies, hepatitis and chicken pox and other

preventable viral diseases. Advantages and disadvantages of cell culture.

The primary advantages of undertaking cell culture are:

1. Cell culture is economical since it is carried out in vitro (i.e. in culture flasks or dishes). It requires small portions of reagents and media unlike in vivo techniques that require a whole organism.
2. Homogenous cells are produced since the growth environment can be controlled in vitro.
3. The physiology and biochemistry of cells can be studied and manipulated in vitro.
4. In cell culture techniques there are no ethical, moral or legal issues as is the case in experimentation that involves the use of a whole animal.
5. Cells in a culture flask/dish can be exposed to chemicals or drugs directly.

The main disadvantages with cell culture are:

1. Cell culture techniques are usually capital intensive. They should be undertaken only when necessary.
2. Cells in cell culture flasks are denied of some in vivo materials such as hormones and other supporting structures that the isolated cell interact with in vivo.
3. Success in cell culture techniques requires expertise to know the behaviour of cells in culture. The aseptic techniques involved usually takes some time to learn.
4. The artificial condition in vitro may cause the cells to produce different substances (e.g. proteins) from the ones they produce in vivo.
5. It is almost impossible to reproduce an in vivo process in an in vitro technique like cell culture.

Contamination of cell cultures. Contamination is a great enemy in the cell culture laboratory. Thus all aseptic techniques must be dutifully followed in order to knock out all sources of contamination in the cell culture. Since contamination by microbes is a major factor in most cell culture techniques it is critical to ensure sterility and/or aseptic techniques at every stage of the experiment in order to get optimum results. Aseptic techniques in the cell culture laboratory ensure that all cell culture protocols are performed to a standard that will prevent contamination from microorganisms (bacteria, fungi and mycoplasmas inclusive) and cross-contamination with other cell lines. Aseptic techniques are all the precautionary measures taken during an experiment to avoid contamination of the work and the researcher. It ensures amongst other things the maintenance of strict sterility in the course of the research. Even though an absolute sterility could not be observed in most cell/tissue culture techniques, it is critical that the researcher imbibe and carry out all the necessary aseptic measures required for that particular experiment in order to avoid the introduction of exogenous or environmental organisms into the cell culture flasks.

Hence, having a cell culture without any form of contamination is paramount in the cell culture laboratory. Such success is usually achieved when the laid down principles and aseptic techniques for undertaking a cell culture procedure are conscientiously followed. A successful cell culture technique depends on a number of factors. These factors are highlighted here:

- The quality of cell lines you are working with.
- The quality of reagents and cell culture media used.
- The aseptic technique used.
- The quality of the laboratory equipment used and their operation.
- The experience of the researcher.

When these factors are met and made available prior to undertaking cell culture experimentation, it is expected that the cell culture technique will be successful.

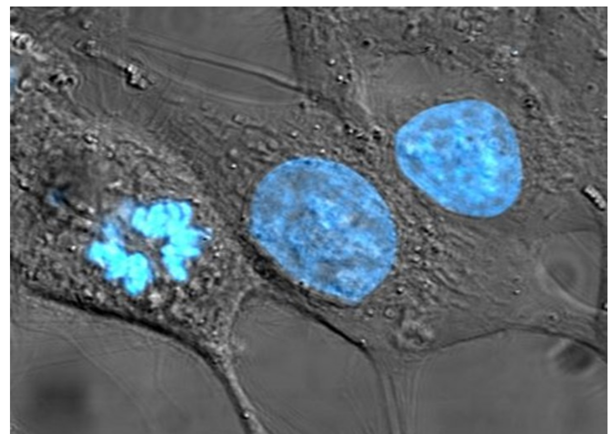

Figure 6 – HeLa cells stained with Hoechst 33258 stain (source: Creative Commons Linrary)

Use of antibiotics in cell development. Bacterial contamination of cells was one of the major threats encountered in the culturing of animal cells in the cell culture laboratory. Microorganisms are naturally ubiquitous. The ever-present nature of microbial organisms should be considered and eliminated as much as possible whenever any cell culture protocol is being con-

templated. Cells can be easily contaminated in the cell culture laboratory especially during collection and passaging. Microbial contamination in the cell culture laboratory should therefore be taken seriously and avoided as much as possible because it can affect the healthiness of the resulting cell lines. The microorganisms that usually affect cell cultures and freshly isolated cells, tissues or organs are: mycoplasmas, bacteria, and fungi. Antibiotics is therefore used and applied during the collection, transportation and the dissection of organs or tissues prior to establishing and getting the primary cell culture. The antibiotics help to eliminate any form of microbial contamination of cells, tissues or biopsies. Decontamination of contaminated cells or their total discarding should be adopted whenever cells are contaminated so as to avoid spreading to other uninfected cells. Antibiotics should be eliminated once the primary cells have been established because long contact of antibiotics with cells can affect some vital eukaryotic cells.

CONCLUSSIONS

Careful physical and microscopic examination of cells can help to detect infections by bacteria and fungi. But this is not the case for mycoplasmas which require more specific screening test for mycoplasmas (which are one of the most serious contaminations in the cell culture lab). Because of the several disadvantages which they portend in cell (tissue) culture experimentations, antibiotics are rarely used for cell culture techniques. The availability and versatility of the biosafety laminar flow cabinet (i.e. the hood) which provides a sterile environment to perform tissue culture experiments have relegated the use of antibiotics in cell culture techniques. Antibiotics may encourage poor aseptic technique and they may also cover mycoplasma infections in cell culture flasks or bottles. Some antibiotics may cross-react with mammalian cells, and thus they may impede the result of the tissue culture experiments when used to prevent contamination of the cells. These reasons and some others have restricted the usage of antibiotics in most cell (tissue) culture experiments. However, the usage of antibiotics as a preventive measure especially to contain bacterial and/or microbial contamination in cell (tissue) culture flasks is not entirely abandoned. The hood should be thoroughly checked and cleaned with 70 % alcohol to reduce contamination within it.

REFERENCES

1 Alberts B. M. Molecular Biology of the Cell /B. M. Alberts, A. Johnson, J. Lewis. – Garland; New York, 2002. – 212 p.

2 Baum C. Side effects of retroviral gene transfer into hematopoietic stem cells /C. Baum, J. Düllmann, Z. Li //Blood. – 2003. – V. 101 (6). – P. 2099-2114.

3 Cabrera C. M. Identity tests: determination of cell line cross-contamination /C. M. Cabrera, F. Cobo, A. Nieto //Cytotechnology. – 2006. – V. 51 (2). – P. 45-50.

4 Cantor J. R. Physiologic Medium Rewires Cellular Metabolism and Reveals Uric Acid as an Endogenous Inhibitor of UMP Synthase /J. R. Cantor, M. Abu-Remaileh, N. Kanarek //Cell. – 2017. – V. 169 (2). – P. 258-272.

5 Chowdhury F. Soft substrates promote homogeneous self-renewal of embryonic stem cells via downregulating cell-matrix tractions /F. Chowdhury, Y. Li, Y. C. Poh //PLOS ONE. – 2010. – V. 5 (12). – e15655.

6 Cooper G. M. The cell: A Molecular Approach /G. M. Cooper, R. E. Hausman //Third edition. - ASM Press, 2004. – P. 67.

7 Davis J. M. Basic Cell Culture, A Practical Approach. – Oxford University Press, Oxford, UK, 2002. – 230 p.

8 Edmondson R. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors /R. Edmondson, J. J. Broglie, A. F. Adcock //Assay and Drug Development Technologies. – 2014. – V. 12 (4). – P. 207-218.

9 Freshney R. I. Culture of Animal Cells, a Manual of Basic Technique, /R. I. Freshney, N. J. Hoboken, J. Wiley. – NY, 2005. – 124 p.

10 Hayflick L. A brief history of the mortality and immortality of cultured cells //The Keio Journal of Medicine. – 1998. – V. 47 (3). – P. 174 -182.

11 Lagziel S. Inferring cancer dependencies on metabolic genes from large-scale genetic screens /S. Lagziel, W. D. Lee, T. Shlomi //BMC Biol. – 2019. – V. 17 (1). – P. 37.

12 Mather J and Barnes D (). Animal cell culture methods, Methods in cell biology /J. Mather, D. Barnes. – San Diego, 1998. – 456 p.

13 Nguyen H. T. Genetic and epigenetic instability in human pluripotent stem cells /H. T. Nguyen, M. Geens, C. Spits //Human Reproduction Update. – 2012. – V. 19 (2). – P. 187-205.

14 Ou D. Promiscuous T-cell recognition of a rubella capsid protein epitope restricted by DRB1*0403 and DRB1*0901 molecules sharing an HLA DR supertype /D. Ou, L. A. Mitchell, D. Décarie //Human Immunology. – 1998. – V. 59 (3). – P. 149-157.

15 Qian L. Improving the expansion and neuronal differentiation of mesenchymal stem

cells through culture surface modification /L. Qian, W. M. Saltzman //Biomaterials. – 2004. – V. 25 (7-8). – P. 1331-1337.

16 Vande Voorde J. Improving the metabolic fidelity of cancer models with a physiological cell culture medium /J. Vande Voorde, T. Ackermann, N.| Pfetzer. – Sci Adv. – 2019. – V. 5 (1). – eaau7314.

17 Voigt N. Methods for isolating atrial cells from large mammals and humans /N. Voigt, C. M. Pearman, D. Dobrev //Journal of Molecular and Cellular Cardiology. – 2015. – V. 86. – P. 187-198.

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Цель настоящей работы – дать студентам, врачам, магистрам фундаментальное представление о клеточных технологиях.

Культура клеток – важный метод как в клеточной, так и в молекулярной биологии, поскольку он обеспечивает лучшую платформу для изучения нормальной физиологии и биохимии клеток.

Причина сосредоточения внимания на системах клеточных культур заключается в том, что они являются незаменимыми инструментами для фундаментальных исследований и широкого спектра клинических исследований *in vitro.* В статье дается критический анализ: как положительные, так и отрицательные аспекты клеточных технологий.

Клеточные культуры могут быть использованы для имитации условий в живом организме. К недостаткам можно отнести то, что культура клеток все же не абсолютная модель живого организма, а лишь напоминает условия жизни живых организмов. Еще один недостаток связан с загрязнением клеточной культуры; их можно разделить на химические и биологические загрязнители.

Работа с культурой клеток – это финансово затратный трудоемкий процесс: требует наличия современного оборудования, профильного помещения, хорошо обученного персонала.

В статье подробно рассматриваются вопросы применения клеточных культур в медицине, фармацевтическом производстве и биологии

Ключевые слова: клетки, жизнь, условия, культура клеток, лабораторная техника

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ЖАСУША ТЕХНОЛОГИЯЛАР НЕГІЗДЕРІ

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Бұл жұмыстың мақсаты студенттерге, дәрігерлерге, магистранттарға жасуша технологиялары туралы негізгі түсінік беру. Жасуша культурасы - бұл жасушалық және молекулалық биологияда маңызды әдіс болып табылады, өйткені ол жасушалардың қалыпты физиологиясы мен биохимиясын зерттеудің ең жақсы платформасын ұсынады. Жасушалық культуралардың жүйелеріне назар аударудың себебі, олар іргелі зерттеулер мен in vitro клиникалық зерттеулерінің кең спектрі үшін таптырмайтын құрал болып табылады. Мақалада сыни талдау келтірілген: жасушалық технологияның оң және теріс аспектілері. Жасуша культураларын тірі ағзадағы жағдайларын модельдеу үшін пайдалануға болады. Кемшіліктерге жасуша культурасы тірі ағзаның абсолютті моделі емес, тек тірі организмдердің өмір сүру жағдайларын еске салатындығын жатқызуға болады. Тағы бір кемшілік жасуша культурасының ластануымен байланысты; оларды химиялық және биологиялық ластаушы заттарға бөлуге болады. Жасушалық культурамен жұмыс жасау қаржылық тұрғыдан көп шығынды талап ететін процесс: ол үшін заманауи жабдықтар, профильді бөлмелер және жақсы дайындап оқытылған қызметкерлер қажет етеді.

Мақалада жасуша культуралары медицинада, фармацевтикалық өндірісте және биологияда қолданылуы егжей-тегжейлі қарастырылады.

Кілт сөздер: жасушалар, тіршілік, жағдайлар, жасуша дақылы, зертханалық технология