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Cell Culture: An insight View

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ABSTRACT

Cell culture is in a limelight for past two decades because of its demand by pharmaceutical companies and cell biologist. Applicability of cell culture can vary from normal cell function to cancer treatment. It is a firm belief that cell culture will be the future trend form drug development to organ development. Hence it is inevitable for us to have knowledge on cell culture. Present article throws a light on basic idea on cell culture.

Key words: cell culture, normal cell function, cancer treatment, pharmaceutical companies.

INTRODUCTION

Cell culture is the process by which cells are grown under controlled conditions generally outside the biological environment. The history dates back to Sydney ringer who developed a salt solution containg various ion concentrations to maintain the isolated animal heart. The techniques advanced in the early 40's for the preparation of vaccines.

Several developments occurred that made cell culture widely available as a tool for scientists. First, there was the development of antibiotics that made it easier to avoid many of the contamination problems. Second was the development of the techniques, such as the use of trypsin to remove cells from culture vessels, necessary to obtain continuously growing cell lines. Third, using these cell lines, scientists were able to develop standardized, chemically defined culture media that made it far easier to grow cells. These three areas combined to allow many more scientists to use cell, tissue and organ culture in their research[1-4]

Contemporary cell culture terminology

The culture of whole organs or intact organ fragments with the intent of studying their continued function or development is called *Organ Culture*. When the cells are removed from the organ fragments prior to, or during cultivation, thus disrupting their normal relationships with neighboring cells, it is called *Cell Culture*.

A primary cell cultureis a culture that is taken directly from cells and tissues oforganisms. A primary culture may be regarded as such until it is successfully subcultured for the first time.

A cell line(established or transformed cell culture) arises from a primary culture at the time of the first successive successful subculture. The term, cell line, implies that cultures from it consist of numerous lineages of cells originally present in the primary culture. The terms, finite, or continuous, are used as prefixes if the status of the culture is known. If not, the term line will suffice.

A finite cell line is generally diploid and, inthis case, no less than 75 per cent of all the cells

must be of the same standardkaryotype as the parent species & its lifespan is approximately 40-50 divisions. A continuous cell line derives from primary cultures or diploid cell lines by transformation processes which are either spontaneous, or induced by viruses, chemical or physical agents.

When a cell line derives from a single cell, it is termed a clonal cell line. Clonal cell lines can be obtained by several techniques as from primary cultures, diploid cell lines &established cell lines. They may not necessarily be homogeneous populations and only frequent cloning can keep culture heterogeneity to a minimum[2,5,6]

A cell strain(extended or multi passage culture) derives either from a primary culture or a cell line by the selection or cloning of cells having specific properties or markers. It is necessary for the properties or markers to persist during subsequent cultivation[2,5,6]

Isolation of cells

Normal adult tissues, embryo and tumour cells can be used for cell culture because they are more easily cultured because they have a higher growth capability and adapt more readily to variations in external factors.

There are two basic methods for obtaining cells for cell culture to obtain primary cell culture. *Explant Culture method* where small pieces of tissue are attached to a glass or treated plastic culture vessel and bathed in culture medium. After a few days, individual cells will move from the tissue explant out onto the culture vessel surface or substrate where they will begin to divide and grow.

The second, more widely used method, speeds up this process by adding digesting (proteolytic) enzymes, such as trypsin or collagenase, to the tissue fragments to dissolve the cement holding the cells together. This creates a suspension of single cells that are then placed into culture vessels containing culture medium and allowed to grow and divide. This method is called *Enzymatic Dissociation*[7]

Maintanence of cells in a culture

Three cell culture conditions that influence cell growth are culture medium, culture substrate, and physiochemical variables.

Culture media

Cell culture media are complex mixtures of salts, carbohydrates, vitamins, amino acids, metabolic precursors, growth factors, hormones, and trace elements. The requirements for these components vary among cell lines and these differences are partly responsible for the extensive number of medium formulations. Carbohydrates are supplied primarily in the form of glucose. In some instances, glucose is replaced with galactose to decrease lactic acid build-up as galactose is metabolized at a slower rate. Other carbon sources include amino acids (particularly L-glutamine) and pyruvates.

Cultural media can be broadly classified into serum and non-serum based media. Non-serum based is the most commonly used media as it is less contaminated. Eagle's Minimum Essential Medium, Dulbecco's Modified Eagle's Medium, Iscove's Modified Dulbecco's Medium, Hybri-Care Medium, McCoy's 5A and RPMI-1640, Ham's Nutrient Mixtures, DMEM/F12 Medium are commercially available[8,9]

Culture substrate

Culture vessels provide a contamination barrier to protect the cultures from the external environment while maintaining the proper internal environment. For anchorage-dependent cells, the vessels provide a suitable and consistent substrate for cell attachment. Other characteristics of vessels include easy access to the cultures and optically clear viewing surfaces.

Earlier all culture vessels were made of glass but ended up with cons of for heavy weight, expense, labor intensive cleaning, and poor microscopic viewing compared to plastic. By the 1960s, surface treatment techniques were developed for polystyrene, allowing plastic vessels to replace glass for most cell culture applications[8,9]

Physico chemical variables

In addition to nutrients, the medium helps maintain the pH and osmolality in a culture system. The pH is maintained by one or more buffering

systems such as CO, /sodium bicarbonate, phosphate, and HEPES[8,9]

Passaging cells

As cells grow it usually follow a characteristic growth pattern composed of four phases: Lag, log/exponential, stationary/plateau and decline. To ensure viability, genetic stability, and phenotypic stability, cell lines need to be maintained in the exponential phase. This means that they need to be subcultured on a regular basis before they enter the stationary growth phase.

To ensure that the characteristics of your cell line remain constant, maintain the cells in the same medium, serum, and supplements with the same subculturing regimen used to establish the culture. Any change to the culturing conditions has the potential to change the characteristics of the cell line[10]

subculturingis mainly done to avoid senescence. cells are detached with the mixture of trypsin-EDTA and some may be mechanically scraped from the vessels with a rubber scraper.

Methods of cell culturing

Monolayer and suspension culturing are the two different methods by which cells are grown. Anchorage-dependent cell lines growing in monolayers need to be subcultured at regular intervals to maintain them in exponential growth. When the cells are near the end of exponential growth (roughly 70% to 90% confluent), they are ready to be subcultured. Most primary cultures, finite cell lines, and continuous cell lines are anchorage dependent and thus grow in monolayers attached to a surface. Other cells, particularly those derived from hematopoietic or certain tumortissues, are anchorage independent and grow in suspension. Both have their own advantages and limitations. [11,12]

3D cell culturing is a recent advancement in this field. They are useful in drug discovery, cancer biology and regenerative medicine. 3D growth can be done in nanoparticle facilitated magnetic levitation, gel matrices scaffolds and hanging drop plates. MLM promotes cell interaction by levitating the cells upto the air/liquid interface of a standard petridish. The assembly consist of magnetic ion oxide nano particles, gold nano particles and polymer poly lysine. It is scalable with the capacity and capability of culturing 500 to millions of cells or from single dish to high through put low volume systems[13,14]

Advantages

1. Primary cell cultures have morphological and biochemical characteristics that are more similar to those of the original tissue karyotype.
2. Cell lines offer the advantage of being more homogeneous and standardized than primary cultures. They are well optimised,easy to cultivate and reproducible results are easier to obtain.
3. Cell strains have the advantage of being more homogeneous populations from the point view of selected characteristics.

Limitations

1. Primary cell cultures have morphological and biochemical characteristics that are more similar to those of the original tissue; however, problems with obtaining reproducible results may negate these advantages. Primary cultures are generally more sensitive to the effects of toxic chemicals than are cell lines because, while exposed, they have also to adapt to culture conditions. The main limitations of primary cultures are low homogeneity and a tendency to rapid loss of specialization under culture conditions.
2. On the other hand, cell lines may be quite different from the original tissue due to the fact that established cell lines have undergone a number of transformations.
3. Cell strains same disadvantages as cell lines from which they derive.

Cell culture contamination

Eliminating contamination from a cell line is time consuming and does not always work. Discarding the culture and starting over is preferred. However, if the cells are unique and irreplaceable, one should first identify the contaminant and select a suitable antibiotic for treatment. It is best to test the contaminating microbe for its antibiotic

sensitivity prior to treatment; this allows for a shorter treatment time and limits exposure of the cell line to potentially damaging reagents.

The cells are cultured for 1 to 2 weeks in the presence of the antibiotic, and then cultured without antibiotic for 1 to 2 months. At this point, the line should be retested with a very sensitive test method to make sure that the culture is clean. Periodic retesting should be employed to make sure that the contaminant does not reappear. Since antibiotics may be toxic to cells, a selected population that no longer exhibits qualities of the parental line may result. It may be necessary to examine the cured culture to determine if it is sufficiently similar to the original line[15,16]

Preserving cell cultures[17]

Most cell cultures can be stored for many years, if not indefinitely, at temperatures below –130°C (cryopreservation). Advantages of preserving cell culture includes

1. Generation of safety stocks to ensure against loss of the culture from equipment failures or contamination by microorganisms or other cell lines.
2. Elimination of the time, energy, and materials required to maintain cultures not in immediate use.
3. Preservation of cells with finite population doublings (that will ultimately senesce).
4. Insurance against phenotypic drift in the culture due to genetic instability and/or selective pressure.
5. Creating a standard reagent to be used for a series of experiments.

Uses of cell cultures[3,18]

Basic tissue functioning

- Cell cultures provide a good model system for studying basic cell biology and biochemistry.
- Helps in gaining knowledge on cell aging.

Pharmaceutical companies

- Cultured cells are widely used alone or in conjunction with animal tests to study the effects of new drugs, cosmetics and chemicals on survival and growth in a wide variety of cell types.
- Large scale production of cells that have been genetically engineered to produce proteins that have medicinal or commercial value. These include monoclonal antibodies, insulin, hormones, etc.
- Toxicity testing is an another important field in pharmaceutical companies where they use cell culture to learn the toxicity of the drug.

Cancer research

- Since both normal cells and cancer cells can be grown in culture, the basic differencesbetween them can be closely studied.
- In addition, it is possible, by the use of chemicals,viruses and radiation, to convert normal cultured cells to cancer causing cells. Thus, themechanisms that cause the change can be studied.
- Cultured cancer cells also serve as a testsystem to determine suitable drugs and methods for selectively destroying types of cancer.

Virology

- Cell cultures are also widely used in the clinical detection and isolation of viruses, as well as basic research into how they grow and infect organisms.
- One of the earliest and major uses of cell culture is the replication of viruses in cell cultures (in place of animals) for use in vaccine production.

Use of cells as replacement tissues and organs usually termed as Tissue engineering

Genetic engineering, counselling and therapy

- The ability to transfect or reprogram cultured cells with new genetic material (DNA and genes) has provided a major tool to molecular biologists wishing to study the cellular effects of the expression of theses genes (new proteins).
- Amniocentesis, where cells of foetus can be examined for abnormalities in the chromosomes and genes using karyotyping, chromosome painting and other molecular techniques.

- Cells can be removed from a patient lacking a functional gene and the missing or damaged gene can then be replaced. The cells can be grown for a while in culture and then replaced into the patient.

CONCLUSION

Animal cell culture has undergone numerous changes from investigative procedure to drug developmental procedure. Though it has vast number of uses, it is still in the beginning stage. With introduction of genetic therapy, engineering and modifications, cell culture is entering in to a new era. Thus a basic knowledge on the cell culture is a must for a dentist.

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