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Monolayer and Micro carrier in Research Scale Applications

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Abstract

Cell culture is the process by which prokaryotic, eukaryotic or plant cells are grown under controlled conditions. But in practice it refers to the culturing of cells derived from animal cells. Cell culture was first successfully undertaken by Ross Harrison in 1907. Roux in 1885 for the first time maintained embryonic chick cells in a cell culture. The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been already established. A wide range of cells have been cultured on micro carriers. For instance, cells from invertebrates, from fish, birds and cells of mammalian origin have been cultivated on micro carriers. Transformed and normal cell lines, fibroblastic and epithelial cells and even genetically engineered cells have been cultivated on micro carriers for various biological such as for the production of immunological like interferon's, interleukins, growth factors etc. Cells cultured on micro carriers also serve as hosts for a variety of viruses that are used as vaccines like foot and mouth disease or rabies.

Index Terms: 1.Cell culture, 2.Micro carriers, 3.Fibroblastic, 4.Interferon's, 5.Genetically, 6.Vaccines, 7.Interleukins.

History of Animal Cell Culture:

Although animal cell culture was first successfully undertaken by Ross Harrison in 1907, it was not until the late 1940's to early 1950's that 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 that plagued earlier cell culture attempts.
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 (such as HeLa cells).
Third, using these cell lines, scientists were able to develop standardized, chemically defined culture media that made it far easier to grow cells.

Introduction: Types of Animal Cell Culture

Primary culture:

When cells are surgically removed from an organism and placed into a suitable culture environment, they will attach, divide and grow. This is called a Primary Culture. There are two basic methods for doing this.

First, for Explant Cultures, small pieces of tissue are attached to a glass or treated in a plastic culture vessel and bathed in culture medium. After a few days, individual cells will move from the tissue explant 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.

Cell Line

After the first subculture, the primary culture becomes known as a cell line or sub-clone. Cell lines derived from

primary cultures have a limited life span (i.e., they are finite; see below), and as they are passaged, cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population.

Cell strain

If a subpopulation of a cell line is positively selected from the culture by cloning or some other method, this cell line becomes a cell strain. A cell strain often acquires additional genetic changes subsequent to the initiation of the parent line.

Different modes of culture are represented from left to right. First an organ culture on a filter disk on a triangular stainless steel grid over a well of medium, seen in section in the lower diagram. Second, explant cultures in a flask, with section below and with an enlarged detail in section in the lowest diagram, showing the explant and radial outgrowth under the arrows. Third, a stirred vessel with an enzymatic disaggregation generating a cell suspension seeded as a monolayer in the lower diagram. Fourth, a filter well showing an array of cells, seen in section in the lower diagram, combined with matrix and stromal cells.

Morphology of Cells in Culture

Cells in culture can be divided in to three basic categories based on their shape and appearance (i.e., morphology).

Fibroblastic cells

Fibroblastic (or fibroblast-like) cells are bipolar or multi-polar, have elongated shapes and grow attached to a substrate.

Epithelial-like cells

Epithelial-like cells are polygonal in shape with more regular dimensions, and grow attached to a substrate in discrete patches.

Lymphoblast-like cells

Lymphoblast-like cells are spherical in shape and usually grown in suspension without attaching to a surface.

Cell culture procedures

The following important conditions must be satisfied to achieve successful cell culture:

· Incubation temperature should be 36°C.

·The pH for growth should be between 7.2 and 7.4.

The levels of glucose and L-glutamine can influence cell growth, and correct levels for each cell line should be checked before attempting to put it into culture (typical levels for glucose and L-glutamine are 1-4 mM and 2 mM, respectively). A range of inorganic ions, amino acids and vitamins are essential for cell survival and will usually be included in basal growth media from proprietary sources. Both oxygen and carbon dioxide are essential and are provided either as a mixture of CO2 and air supplied to the culture vessel or by sealing the vessel tightly to retain the CO2 produced by cell metabolism.

Aseptic technique

Skill in aseptic technique is important to maintain sterility during media preparation and cell cultivation procedures. Furthermore, it is a vital component in ensuring operator protection from infectious agents that may be present in culture materials. Some important elements in aseptic technique are:

· Sterilize all glassware for handling cell cultures and media

· Avoid splashes, spills and aerosols.

· Avoid liquid transfer by pouring.

· When adding (or replacing) medium, never touch the neck of the culture flasks with the bottle containing the medium or use the same pipette to transfer medium to more than one bottle. Ideally, aliquot the total amount of medium required for each batch of culture bottles being handled and store the remainder at 4-8°C. Dedicate separate medium for each cell line.

· Separate clean and contaminated materials in the BSC II.

· Minimize exposure of sterile media and cell cultures to open air (even within the BSC II).

· Perform any final preparation of sterile media (i.e. addition of serum or other additives) before dealing with cell cultures.

Because of the risks of contamination and cross-infection, cell culture in the virus diagnostic laboratory is best carried out in closed vessels, usually screw-capped tubes and flat-sided bottles. WHO does not recommend the use of 24-well plates for the isolation of polioviruses from stool specimens as this method is inappropriate to conditions encountered in many laboratories of the Global Polio Laboratory Network. Cultures are initially set up in growth medium supplemented with 10% serum. Once the cells have formed a confluent monolayer, cultures are changed to maintenance medium which is designed to maintain cultures in a healthy state for as long as possible without stimulating growth; this is achieved by reducing the serum content, usually to 2%.

Preparation of glassware

Due to the difficulty of cleaning and recycling glassware to culture quality, many laboratories have resorted to using disposable cell culture plastic ware. If a laboratory chooses to use glassware, however, it must ensure that all glassware is meticulously cleaned and sterilized so that cell cultures will not be affected by traces of proteinaceous material, detergent, pyrogens, water deposits and other residual materials which may get deposited on the glassware. Glassware cleaning protocols should be developed along the lines of the following procedures:

· Use care in handling glassware as most breakages occur during the cleaning process.

· Before cleaning, decontaminate glassware by autoclaving or soaking overnight in chlorine solution (0.5%).

· Decontaminate pipettes in a container containing chlorine.

· Rinse all glassware as soon as possible after use.

· Store soiled items in water containing a disinfectant or cleanser to avoid drying and making items harder to clean.

· Use 7-X, DECON or similar detergent for thorough cleaning of all laboratory glassware. These detergents are easily rinsed from glassware without leaving residues. (DO NOT use domestic dishwashing liquid detergent under any circumstances.)

· Clean glass by scrubbing with a brush. Periodically inspect brushes for wear to avoid scratching glass.

· Thoroughly rinse items in tap water, followed by at least 5-7 changes of distilled or deionized water. Even the smallest residual amounts of cleansers, disinfectants or acids can affect the growth of cell cultures.

· Dry glassware on racks or peg boards and inspect after drying. If glassware is hazy, has a film or blotches are evident, then additional cleaning is required before use.

· Sterilize cell culture glassware using a hot air oven at 180°C for three hours to destroy pyrogens. Non-glass components which may not withstand 180°C should be sterilized by alternate methods such as autoclaving, and re-assembled aseptically.

Chromic acid wash: Some heavily soiled glassware may require vigorous methods to clean and traditionally this has required the use of chromic acid (10% potassium dichromate in 25% sulfuric acid). Chromic acid, however, is a hazardous substance, with safety and environmental concerns. There are effective commercially available substitutes to chromic acid which include: Fisher product, Contrad 70 or VWR Scientific products, Chem-Solv, phosphate-free formulations of RBS-35, PCC-54 and Nochromix (also supplied by Fisher).

If chromic acid must be used, follow all normal safety precautions for using concentrated acids and acid solutions. As with any other cleaning process, all cleaning solutions must be completely rinsed from the glassware through copious changes of tap water followed by several changes of distilled water.

Selection of cell culture systems

Many cell culture systems support the growth of polioviruses and other enteroviruses. Regional reference laboratories (RRL) are advised to obtain cell cultures from the official collections. Requests for these cell lines should be submitted to IVB/VAM, WHO, Geneva.
National poliomyelitis laboratories can in turn apply to their designated RRL for supplies of these cell lines. As soon as possible after the receipt of cell cultures, a cell bank should be established in liquid nitrogen, or if this is not available, in a mechanical freezer at -70°C or lower. Cells stored at -70°C will not remain viable for very long periods and aliquots should be resuscitated every 4-6 months, passaged to build up numbers, and stored again at -70°C.

Preparation of cell culture systems
Cells should be received with documented evidence for the key characteristics relating to the quality of cell cultures as described above. In handling cell cultures, laboratory personnel must be concerned not only with preventing microbial contamination of the cultures, but also with avoiding contamination of the working environment with cell culture materials. All cultures must be considered potentially hazardous, whether inoculated or un-inoculated. After use all cultures and their fluids should be decontaminated by autoclaving. Cross-contamination between different cell types, especially continuous cell lines, is an ever-present hazard. To avoid this, different cell lines should never be processed at the same time. All working areas should be thoroughly cleaned between the preparations of different cell types.
Cell culture media employed in virology can be divided into two main categories, growth media and maintenance media. Growth media (GM), high in serum content (usually 10%), promote rapid cell growth. After a monolayer has formed and prior to inoculation with virus, the growth medium is removed and replaced with maintenance medium.
Maintenance media (MM), low in serum content (usually 2%), are intended to keep the cell cultures in a steady state of slow cell replication whilst maintaining cell metabolism during the period of viral replication. Fetal calf serum is the serum of choice: it is good for promoting cell growth and it lacks viral inhibitors. If serum from other sources is used, it must be pre-tested for the presence of inhibitors to the viruses being studied. All sera for cell culture use must be inactivated at 56°C for 30 minutes.

Isolation of cells
Cells can be isolated from tissues for ex vivo culture in several ways. Cells can be easily purified from blood; however only the white cells are capable of growth in culture. Mononuclear cells can be released from soft tissues by enzymatic digestion with enzymes such as collagenase, trypsin or protease, which break down the extracellular matrix.
Alternatively, pieces of tissue can be placed in growth media, and the cells that grow out are available for culture. This method is known asepticplantculture.

Cells that are cultured directly from a subject are known as primary cells. With the exception of some derived from tumours, most primary cell cultures have limited lifespan. After a certain number of population doublings cells undergo the process of senescence and stop dividing, while generally retaining viability. An established or immortalized cell line has acquired the ability to proliferate indefinitely either through random mutation or deliberate modification, such as artificial expression of the telomerase gene. There are numerous well established cell lines representative of particular cell types.

Culture Conditions
Culture conditions vary widely for each cell type, but the artificial environment in which the cells are cultured invariably consists of a suitable vessel containing a substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones, and gases (O2, CO2), and regulates the physicochemical environment (pH, osmotic pressure, temperature). Most cells are anchorage dependent and must be

cultured while attached to a solid or semi-solid substrate (adherent or monolayer culture), while others can be grown floating in the culture medium (suspension culture).

Maintaining cells in culture

Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 370C, 5% CO2for 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 being expressed. Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the growth medium. Recipes for growth media can vary in pH, glucose concentration, growth factors, and the presence of other nutrients. The growth factors used to supplement media are often derived from animal blood, such as calf serum. One complication of these blood-derived ingredients is the potential for contamination of the culture with viruses or prions, particularly in biotechnology medical applications. Current practice is to minimize or eliminate the use of these ingredients wherever possible, but this cannot always be accomplished. Cells can be grown in suspension or adherentcultures. Some cells naturally live in suspension, without being attached to a surface, such as cells that exist in the bloodstream. Adherent cells require a surface, such as tissue culture plastic, which may be coated with extracellular matrix components to increase adhesion properties and provide other signals needed for growth and differentiation. Most cells derived from solid tissues are adherent.

Cell line cross-contamination

Cell line cross-contamination can be a problem for scientists working with cultured cells. Cells used in experiments have been misidentified or contaminated with another cell lines. To prevent cell line cross-contamination, researchers are encouraged to authenticate their cell lines at an early passage to establish the identity of the cell line. Authentication should be repeated before freezing cell line stocks, every two months during active culturing.

Manipulation of cultured cells

As cells generally continue to divide in culture, they generally grow to fill the available area or volume. This can generate several issues:

Nutrient depletion in the growth media and Accumulation of dead cells.

Cell-to-cell contact can stimulate cell cycle arrest, causing cells to stop dividing known as contact inhibition or senescence.

Cell-to-cell contact can stimulate cellular differentiation.

Among the common manipulations carried out on culture cells are media changes, passaging cells, and transfecting cells. Manipulations are typically carried out in a biosafety hood or laminar flow cabinet to exclude contaminating micro-organisms. Antibiotics (e.g. penicillin and streptomycin) and antifungal (e.g. Amphotericin B) can also be added to the growth media. As cells undergo metabolic processes, acid is produced and the pH decreases. Often, a pH indicator is added to the medium in order to measure nutrient depletion.

Applications of cell culture

Cell culture is one of the major tools used in cellular and molecular biology, providing excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging), the effects of drugs and toxic compounds on the cells and mutagenesis and carcinogenesis. It is also used in drug screening and development and large scale manufacturing of biological compounds (e.g., vaccines, therapeutic proteins). The major advantage of using cell culture for any of these applications is the consistency and reproducibility of results that can be obtained from using a batch of clonal cells.

Model System:

Cell culture are used as model system to study basic cell biology and biochemistry, to study the interaction between cell and disease causing agents like bacteria, virus, to study the effect of drugs, to study the process of aging and also it is used to study triggers for ageing.

Cancer Research

The basic difference between normal cell and cancer cell can be studied using animal cell culture technique, as both cells can be cultured in laboratory. Normal cells can be converted into cancer cells by using radiation, chemicals and viruses. Thus, the mechanism and cause of cancer can be studied. Cell culture can be used to determine the effective drugs for selectively destroy only cancer cells.

Virology

Animal cell cultures are used to replicate the viruses instead of animals for the production of vaccine. Cell culture can also be used to detect and isolate viruses, and also to study growth and development cycle of viruses. It is also used to study the mode of infection.

Toxicity Testing:

Animal cell culture is used to study the effects of new drugs, cosmetics and chemicals on survival and growth of a number of types of cells. Especially liver and kidney cells. Cultured animal cells are also used to determine the maximum permissible dosage of new drugs.

Vaccine Production:

Cultured animal cells are used in the production of viruses and these viruses are used to produce vaccines. For example vaccines for deadly diseases like polio, rabies, chicken pox, measles and hepatitis B are produced using animal cell culture.

Genetically Engineered Protein:

Animal cell cultures are used to produce commercially important genetically engineered proteins such as monoclonal antibodies, insulin, hormones, and much more.

Replacement Tissue or Organ:

Animal cell culture can be used as replacement tissue or organs. For example artificial skin can be produced using this technique to treat patients with burns and ulcers. However research is going on artificial organ culture such as liver, kidney and pancreas. Organ culture techniques and research are being conducted on both embryonic and adult stem cell culture. These cells have the capacity to differentiate into many different types of cells and organs. It is belived that by learning to control the development and differentiation of these cells may be used to treat variety of medical conditions.

Genetic Counseling:

Fetal cell culture extracted from pregnant women can be used to study or examine the abnormalities of chromosomes, genes using karyotyping, and these findings can be used in early detection of fetal disorders.

Genetic Engineering:

Cultured animal cells can be used to introduce new genetic material like DNA or RNA into the cell. These can be used to study the expression of new genes and its effect on the health of the cell. Insect cells are used to produce commercially important proteins by infecting them with genetically altered baculoviruses.

Gene Therapy:

Cultured animal cells can be genetically altered and can be used in gene therapy technique. First cells are removed from the patient lacking a functional gene or missing a functional gene. These genes are replaced by functional genes and altered cells are culture and grown in laboratory condition. Then these altered cells are introduced into the patient. Another method is by using viral vector, functional gene is inserted into the genome of viral vector and then

they are allowed to infect the patient, in the hope that the missing gene will be expressed with the help of the viral vector.

Drug Screening and Development:

Animal cell cultures are used to study the cytotoxicity of new drug. This is also used to find out the effective and safe dosage of new drugs. Now these tests are being conducted in 384 and 1536 well plates. Cell-based assay plays an important role in pharmaceutical industry.

Conclusion

A single cell is the building block for life. The genetic material of each cell in the body - itself composed of 100 trillion cells - holds the secret to inherited diseases, such as Tay Sachs, cystic fibrosis and other complex diseases like heart disease. Tissue culture was first developed in the early 1900's as a method for studying the behavior of cells - free of the variations that might arise in the whole organism - in response to normal and induced experimental stress. Initially, scientists used fragments of tissues, but gradually developed techniques to study the behavior of single cells and changed the name to cell culture.But now cell culture technique plays an important role in research and development of drug discovery and also helps in improving the health and quality of life of patients suffering from dangerous diseases like cancer, genetic disorders.

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