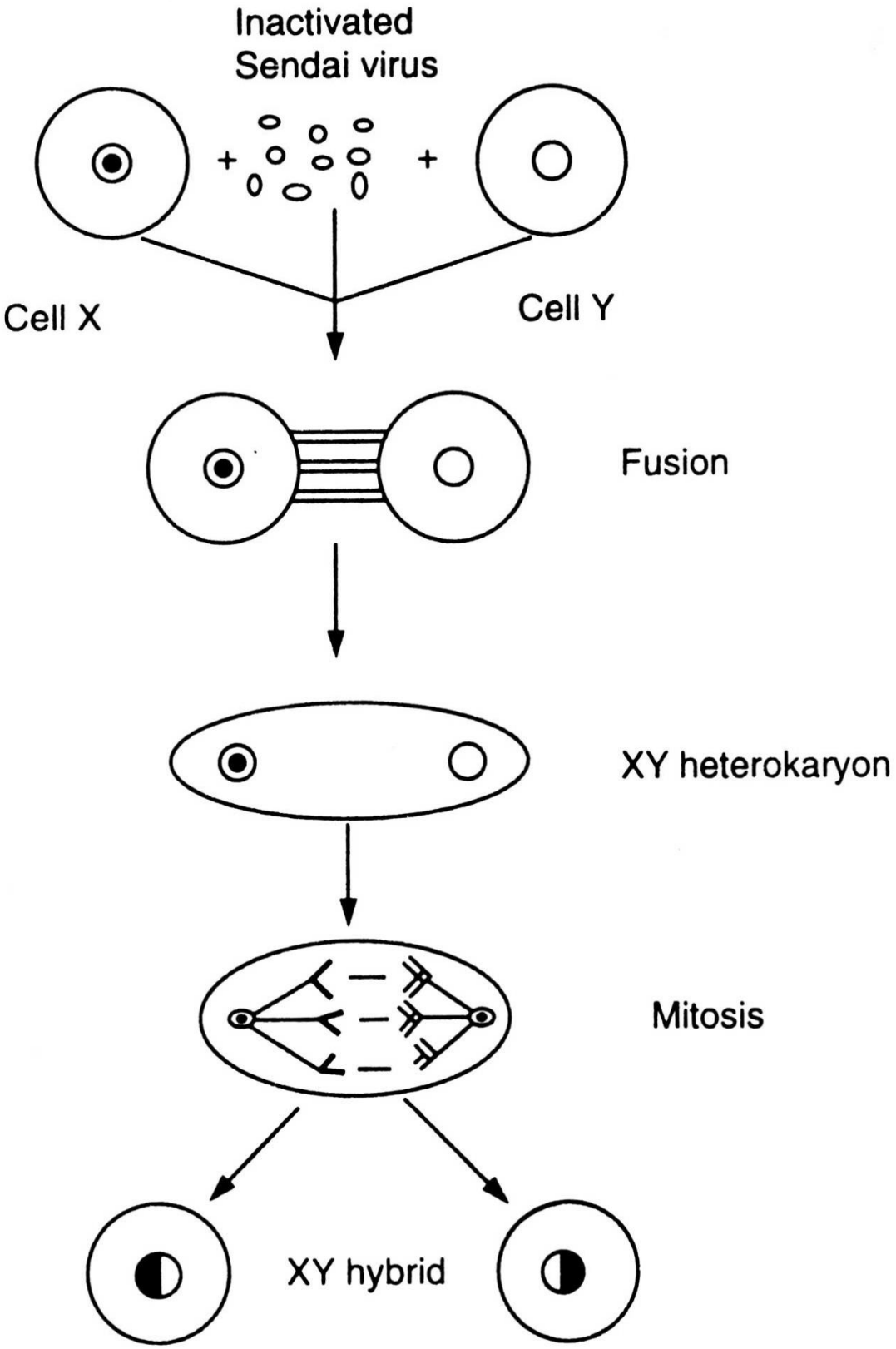
Somatic Cell-fusion

In animals fusion of two different cells and production of a hybrid cell have been successfully achieved. These hybrid cells have significant biotechnological applications in many more areas such as: *(i)* study of control of gene expression and differentiation, *(ii)* gene mapping, *(iii)* malignancy, *(iv)* viral replication, and *(v)* antibody production through hybridoma technology. In 1960s, in France, the hybrid cells were successfully produced from mixed cultures of two different cell lines of mouse.

Moreover, within the body fusion of myoblasts and formation of multinucleate fibers may be exemplified. They can also be allowed to fuse *in vitro* and form heterokaryons. Macrophages fuse around the foreign body or bacterial cells in the tissues. Bone cells are also known to undergo somatic cell fusion. Cells growing in culture are induced by some of the viruses such as 'Sendai virus' to fuse and form hybrids. This virus induces two different cells first to form heterokaryon (Fig. 6.7). During mitosis chromosome of heterokaryons are brought towards two poles which later on fuse to form hybrids. Removal of surface carbohydrates is necessary before establishment of cell fusion. Some chemicals such as polyethylene glycol also induce somatic cell fusion. It is interesting to note that the cells of taxonomically different animals can fuse and form hybrids. This suggests that there is no compatibility between membranes, nuclei, organelles of two different groups of animal cells (Sidebottom and Ringertz, 1984).


Inactivated
Sendai virus
Cell X
Cell Y
Fusion
XY heterokaryon
Mitosis
XY hybrid

CELL AND TISSUE CULTURES:

Introduction

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.

Primary Culture

Primary culture refers to the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate (i.e., reach confluence). At this stage, the cells have to be subculture (i.e., passage) by transferring them to a new vessel with fresh growth medium to provide more room for continued growth.

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.

History

The 19th-century English physiologist Sydney Ringer developed salt solutions containing the chlorides of sodium, potassium, calcium and magnesium suitable for maintaining the beating of an isolated animal heart outside of the body. In 1885 Wilhelm Roux removed a portion of the medullary plate of an embryonic chicken and maintained it in a warm saline solution for several days, establishing the principle of tissue culture. Ross Granville Harrison, working at Johns

Hopkins Medical School and then at Yale University, published results of his experiments from 1907-1910, establishing the methodology of tissue culture.

Types of cell culture:

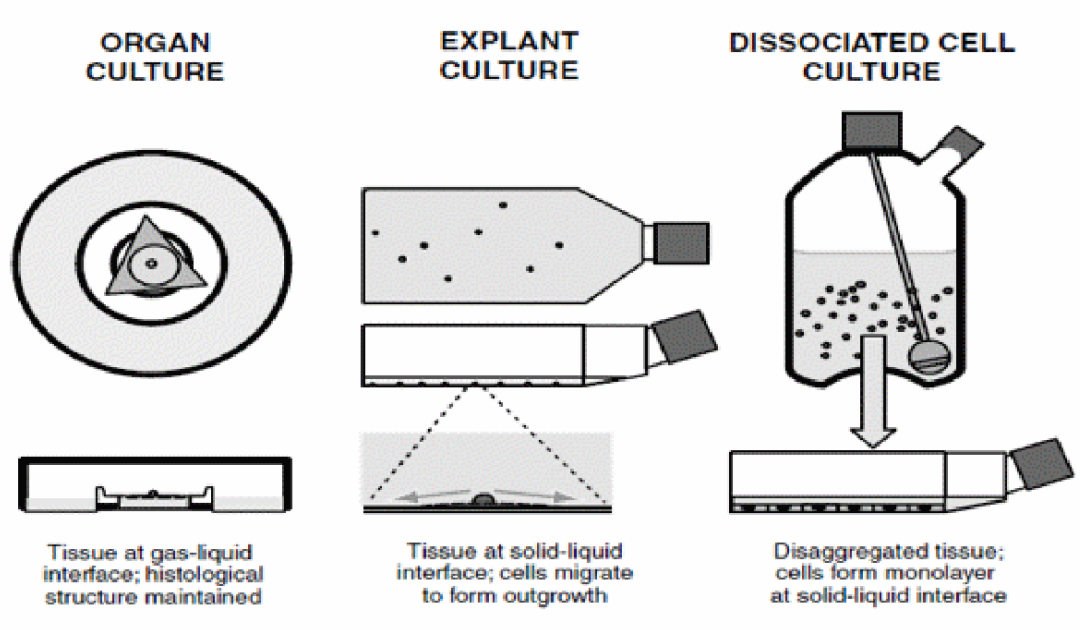


Fig: Types of culture. Different modes of culture are represented from left to right. First an organ culture on a filter disk on a triangular stainless steel grid over a well of medium, seen in section in the lower diagram. Second, explant cultures in a flask, with section below and with an enlarged 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).

1. **Fibroblastic cells**

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

2. **Epithelial-like cells**

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

3. **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:

· Incubationtemperature 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 CO_2 and air supplied to the culture vessel or by sealing the vessel tightly to retain the CO_2 produced by cell metabolism.

1. **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%.

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.

3. 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.

4. 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 vivoculture 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 digestionwith 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 as explantculture.

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 immortalised cell linehas 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 (O_2, CO_2), 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, 37^0C, 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 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 suspensionor 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
- 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 antifungals (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.

Tissue culture is the growth of tissues or cells separate from the organism. This is typically facilitated via use of a liquid, semi-solid, or solid growth medium, such as broth or agar. Tissue culture commonly refers to the culture of animal cells and tissues.

The principal purpose of cell, tissue and organ culture is to isolate, at each level of organization, the parts from the whole organism for study in experimentally controlled environments. It is characteristic of intact organisms that a high degree of interrelationship exists and interaction occurs between the component parts. Cultivation *in vitro* places cells beyond the effects of the organism as a whole and of the products of all cells other than those introduced into the culture. Artificial environments may be designed to imitate the natural physiological one, or varied at will by the deliberate introduction of particular variables and stresses.

Virtually all types of cells or aggregates of cells may be studied in culture. Living cells can be examined by cinephotomicrography, and by direct, phase-contrast, interference, fluorescence, or ultraviolet microscopy. Fixed cells from culture are suitable for cytological, cytochemical, histological, histochemical, and electron microscopical study. Populations of cells from monolayers or suspension cultures are used for nutritional, biochemical, and immunological work.

Organ culture, the cultivation of whole organs or parts thereof, is particularly suitable for studies of development, of inductive interactions, and of the effects of chemical and physical agents upon the physiological functions of specific organs.

Both cell and organ culture have applications in pathology, *e.g.*, for comparative, developmental, and diagnostic studies of tissues from normal and diseased donors, for investigations on carcinogenesis, somatic cell genetic variation, viral susceptibility, etc. Cell cultures are widely used in microbiological studies, for investigations of the effects of radiation, and for screening drugs, especially carcinogenic, mutagenic, and radiomimetic agents.

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