CONTENTS

INTRODUCTION

Tissue culture is the method of 'in vitro' culture of plant or animal cells, tissue or organ – on nutrient medium under aseptic conditions usually in a glass container. Tissue culture is sometimes referred to as 'sterile culture' or 'in vitro' culture. The term "Tissue culture" was coined by Montrose Thomas Burrows. By this technique living cells can be maintained outside the body of the organism for a considerable period. Tissue culture is referred to any multicellular culture with protoplasmic continuity between cells and growing on a solid medium or attached to a substratum and nourished by a liquid medium.

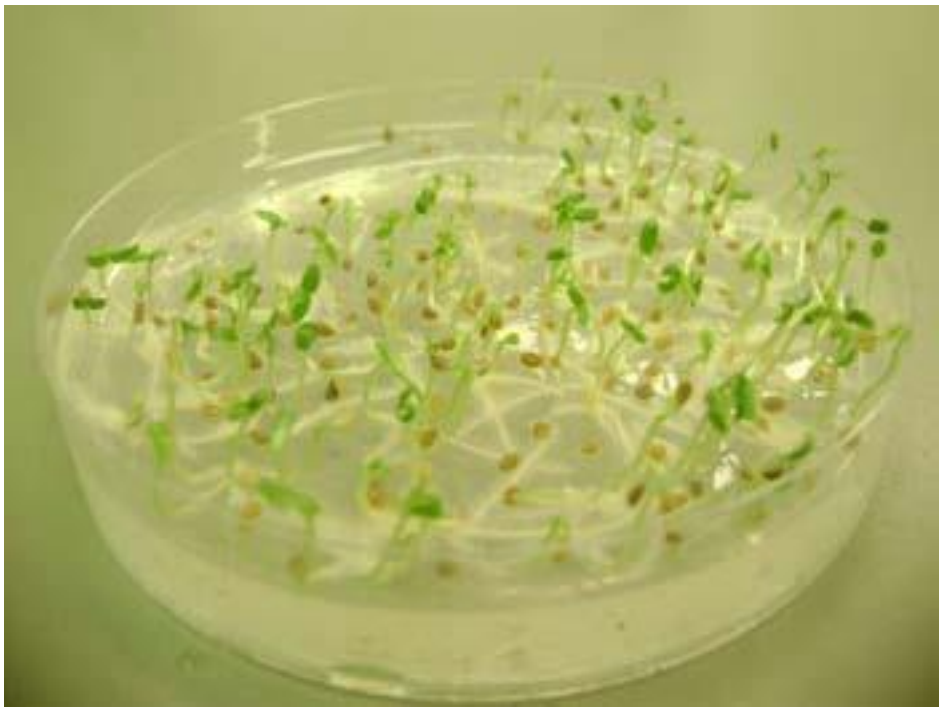
While the term tissue culture may be used for both plant and animal tissues, plant tissue culture is the more specific term used for the culture of plant tissues in tissue culture.

TWO MAIN TYPES OF TISSUE CULTURE

1. Plant tissue culture -Tissue culture is applied in plant research for such purposes as the growing of new plants, which in some cases undergo genetic alterations. Here, the plant of interest is taken through the tissue culture process and grown in a controlled environment.

2. Animal cell culture – In a cell culture technique, cells are removed from an animal or a plant, and grown subsequently in a favourable environment. For animal cell culture the cells are taken from the organ of an experimental animal. The cells may be removed directly or by mechanical or enzymatic action. The cells can also be obtained by previously made cell line or cell strain. Examples of cells used to culture are fibroblast, lymphocytes, cells from cardiac and skeletal tissues etc.

PLANT TISSUE CULTURE



Plant tissue culture is the technique of maintaining and growing plant cells, tissues or organs especially on artificial medium in suitable containers under controlled environmental conditions.

The part which is cultured is called explant, i.e., any part of a plant taken out and grown in a test tube, under sterile conditions in special nutrient media. This capacity to generate a whole plant from any cell/explant is called cellular toti-potency. In fact, the whole plant can be regenerated from any plant part (referred to as explant) or cells. Gottlieb Haberlandt first initiated tissue culture technique in 1902.

Plant Tissue Culture: historical highlights

1902: Haberlandt attempted to the culture mesophyll tissue and root hair cells. This was the first attempt of *in vitro* culture.
1904: Haning attempted to culture excised embryos from mature seeds.
1922: Kotte was successful in obtaining growth from isolated root tips on inorganic media. Robbins reported similar success from root tip and stem tip.
1920-40: First PGR, IAA, discovered by experiments on oat seedlings (Fritz Went).
1934: Used yeast extract (vit B) with inorganic salts to repeatedly culture root tips of tomato.
1935: Importance of B vitamins and PGRs in culture of mesophyll cells.
1936: Haning experiment was repeated with IAA: works !!!
1939: Tobacco crown gall culture, callus obtained: called as Plant Cancer.
1940: WWII. Coconut milk used in plant cultures to obtain heart-shaped embyos.
1950s:Skoog used adenine sulfate to obtain buds on tobacco segments: PGR #2 identified: kinetin
1958: Stewart and Reinert obtained somatic embryos from carrot cells using PGRs.
1950-60s: Botanists turned to plant tissue culture to study plant development.

Conditions Required for Plant Tissue Culture:

There are some important aspects of tissue culture. These are: (A) Aseptic condition, (B) Aeration, (C) Equipments, and (D) Nutrient medium.

A. Aseptic condition -

Tissue culture should be done in completely aseptic condition. Dry heat is used to sterilise equipment's in an incubator. Wet heat sterilization is done in an autoclave at 120°C at 15 lb pressure for 15 minutes. Liquid media, which are unstable at high temperature are sterilised by ultrafiltration. Chemicals, such as alcohol is used to sterilise working area and instruments.

The tissue to be cultured is surface sterilised chemically some of the commonly used sterilising agents are:

(a) 9-10% calcium hypochlorite

(b) 2% sodium hypochlorite solution

(c) 10-12% hydrogen peroxide,

(d) 1-2% bromine water. Some other sterilising agents are: 1% chlorine water, mercuric chloride, silver nitrate, antibiotics etc.

(b) Aeration:

Proper aeration of the tissue in the culture medium is essential. Those tissues, which are cultured on semi-solid medium do not require any special method for aeration. But those tissues, which are cultured in liquid medium require special device for aeration.

(c) Equipments:

Glasswares used for tissue culture should be of borosilicate glass (Pyrex glass), because soda glass may hamper the growth of the tissue.

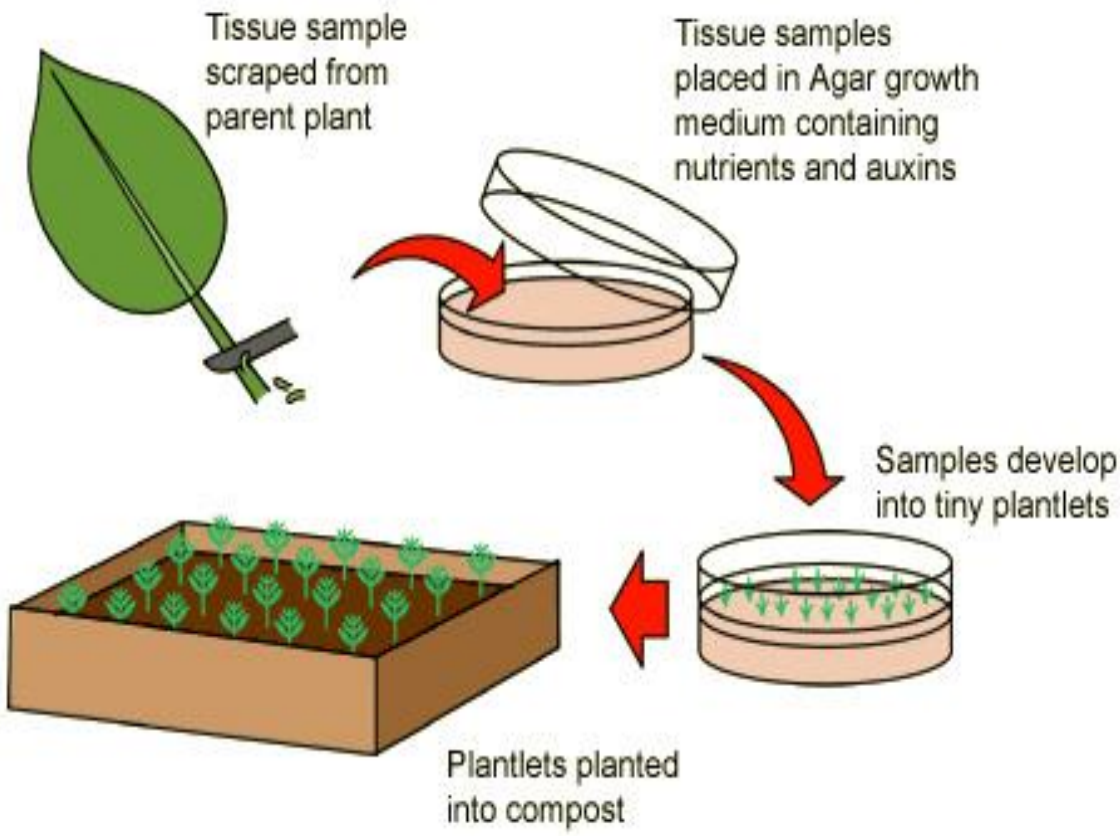
(d) Nutrient media:

Nutrient media required for culture vary with the kind of plant and the purpose of producing the culture. Most of the media contain some inorganic salts of major and minor elements, vitamins and sucrose. Such a medium is called a

basal medium. The pH of the culture medium is maintained between 5.6 – 5.8. If required the medium is solidified with 0.5 – 1% agar. Inorganic substances – Nitrogen is usually added in the form of nitrate or ammonium salt.

Potassium is added as KC1 or KNO_3 or KH_2PO_4. Calcium is added in the form of $CaCl_2.2H_2O$ or Ca $(NO_3)_2.4H_2O$. Magnesium and sulphur are added in the form of magnesium sulphate ($MgS0_4.7H_20$). Phosphorus is obtained from $NaH_2PO_4.H_2O$. Organic substances -2 – 4% sucrose is usually used in a medium. Vitamins are usually required in traces. Thiamine is added as thymine hydrochloride. Nicotinic acid (0.5 mg/1), thiamine (0.1-1 mg/1) and pyridoxine (0.5 mg/1) are usually added to the medium. Most callus cultures require a supply of hormones such as auxin (IAA, NAA and 2-4-D) and Cytokinin (Zeatin, Kinetin and benzyl adenine). Gibberellin is not usually required, but it is needed for apical meristem culture. Ethylene helps differentiation of tracheary elements.

The Process of Plant Tissue Culture



This process involves the use of small pieces of a given plant tissue (plant of interest). Once the tissue is obtained, it is then cultured in the appropriate medium under sterile conditions so as to prevent various types of microorganisms from affecting the process.

The following is a general procedure for plant tissue culture

Medium preparation

- The appropriate mixture (such as the MS mixture) is mixed with distilled water and stirred while

adding the appropriate amount of sugar and sugar mixture. Here, sodium hydroxide or hydrochloric acid is used to adjust the pH - Contents used here will depend on the plant to be cultured and the number of tissues to be cultured.

- Agar is added to the mixture, heat and stirred to dissolve
- After cooling, the warm medium is poured into polycarbonate tubes (to a depth of about 4 cm)
- With lids sitting on the tubes, the tubes are placed in a pressure cooker and sterilized for 20 minutes

Plant preparation

- Cut the plant part in to small pieces (e.g. cauliflower can be cut to florets of about 1cm across). On the other hand, such parts as the African violet leaves can be used as a whole.
- Using detergent and water, wash the plant part for about 20 minutes
- Transfer the plant part in to sterilizing Clorox solution, shake for a minute and leave to sock for 20 minutes
- Using a lid, gently discard the Clorox and retain the plant part in the container and then cap the container

Transferring the plant material to a tissue culture medium

* 70 percent alcohol should be used for the sterilization of the equipment used and containers

- Open the container and pour sterile water to cover half the container
- Cover with a sterile lid again and shake the container for 2 to 3 minutes in order to wash the tissue and remove the bleach
- Pour the water and repeat this three times
- Using sterilized gloves, remove the plant part from the container and on to a sterile Petri dish
- Using a sterile blade cut the plant material to smaller pieces of about 2 to 3 mm across avoiding the parts that have been damaged by bleach
- Using sterile forceps, place a section of the plant in to the medium

Cauliflower - partly submerged in medium with flower bud facing up

Rose with shoots at level with medium surface

African violet leaf laid directly in surface of medium

*depending on the plant used, it is important to check and find out how it should be placed in the medium

Replace the lid/cap and close tightly

This procedure will result in the development of a callus, which then produces shoots after a few weeks. Once the shoots develop, then the plant section may be placed in the right environment (well lit, warmth etc) for further growth.

* Plant materials should be sterilized so as to remove any bacteria or spores that may be present.

For plants, the medium culture acts as a greenhouse that provides the explant with the idea environment for optimum growth. This includes being free of microorganisms, nutrients as well as the right balance of chemicals and hormones. Such media as BAP, TDZ are used while such hormones as IBA and IAA are used to induce growth. Some of the major reasons tissue culture is used for plants include;

- To produce large quantities of a given plant
- To accelerate the production of new varieties of a plant
- To maintain a virus free stock of the plant of interest.

Technique for Plant in Vitro Culture

Micro propagation - This technique is used for the purposes of developing high- quality clonal plants (a clone is a group of identical cells). This has the potential to provide rapid and large scale propagation of new genotypes.

Somatic cell genetics - Used for haploid production and somatic hybridization.

Transgenic plants - Used for expression of mammalian genes or plant genes for various species it has proved beneficial for the engineering of species that are resistant against viruses and insects.

ANIMAL CELL CULTURE

Cell culture - It is the process by which cells are grown under controlled conditions, generally outside their natural environment. After the cells of interest have been isolated from living tissue, they can subsequently be maintained under carefully controlled conditions. The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture

Historical Events in the Development of Cell Culture:

1878: Claude Bernard proposed that physiological systems of an organism can be maintained in a living system after the death of an organism.

1885: Roux maintained embryonic chick cells in a saline culture.

1897: Loeb demonstrated the survival of cells isolated from blood and connective tissue in serum and plasma.
1903: Jolly observed cell division of salamander leucocytes in vitro.
1907: Harrison cultivated frog nerve cells in a lymph clot held by the 'hanging drop' method and observed the growth of nerve fibres in vitro for several weeks. He was considered by some as the father of cell culture.
1910: Burrows succeeded in long-term cultivation of chicken embryo cell in plasma clots. He made detailed observation of mitosis.
1911: Lewis and Lewis made the first liquid media consisted of sea water, serum, embryo extract, salts and peptones. They observed limited monolayer growth.
1913: Carrel introduced strict aseptic techniques so that cells could be cultured for long periods.
1916: Rous and Jones introduced proteolytic enzyme trypsin for the subculture of adherent cells.
1923: Carrel and Baker developed 'Carrel' or T-flask as the first specifically designed cell culture vessel. They employed microscopic evaluation of cells in culture.
1927: Carrel and Rivera produced the first viral vaccine – Vaccinia.
1933: Gey developed the roller tube technique.

Requirements for Animal Cell Culture :

A. **Sterile Work Area –**
This room should be free of "through traffic" and, if possible, equipped with an air flow cabinet which supplies filtered air around the work surface.

B. Incubation Facilities –
In addition to an airflow cabinet and benching which can be easily cleaned, the cell culture laboratory will need to be furnished with an incubator or hot room to maintain the cells at 30-40°C.

C. Refrigerators and Freezer -
Both items are very important for storage of liquid media at 4°C and for enzymes (e.g., trypsin) and some media components (e.g., glutamine and serum) at -20°C. A refrigerator or cold room is required to store medium.

D. Microscopes:
A simple inverted microscope is essential so that cultures can be examined in flasks and dishes.

E. Tissue Culture Ware –
All tissue culture plastic-ware should support cell growth adequately, it is essential when using a new supplier or type of dish to ensure that cultures grow happily in it.

F. **Washing Up and Sterilizing Facilities-**
Availability of a wide range of plastic tissue culture reduces the amount of necessary washing up.

G. Liquid Nitrogen Deep Freezer-

Invariably for continuous and finite cell lines, samples of cultures will need to be frozen down for storage. To achieve slow freezing rates a programmable freezer or an adjustable neck plug or freezing tray for use in a narrow-necked liquid nitrogen freezer can be used.

H. Water Still or Reverse Osmosis Apparatus -

A double distilled or reverse osmosis water supply is essential for preparation of media, and rinsing glassware.

I. Filter Sterilization-

Media that cannot be autoclaved must be sterilized through a 0.22 mm pore size membrane filter.

J. Other Requirements –

(a) Temperature

(b) Culture Media

(c) pH

(d) Osmolality

(e) Buffering

(f) Growth Factors

(g) Antibiotics and Antimycotics

Techniques of Animal Cell Culture

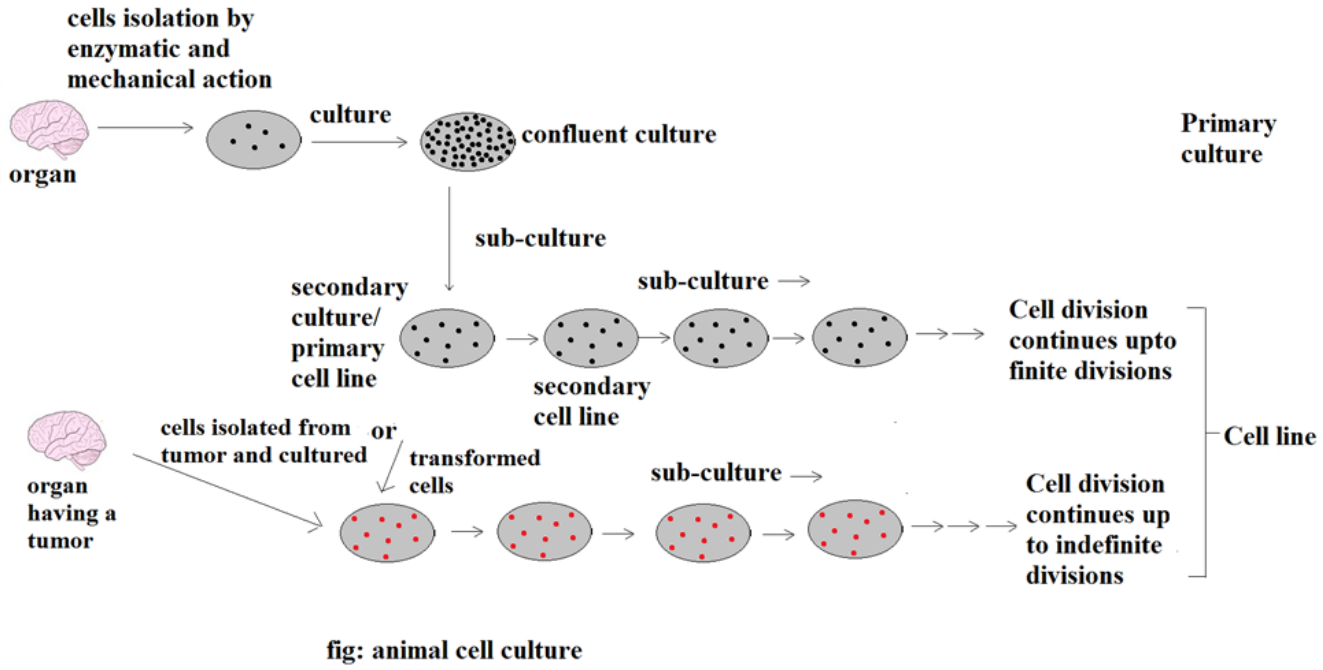


fig: animal cell culture

Unlike plant and microbial cells, animal cells are also cultured in vitro on specific culture media. The steps involved are-

Primary Cell Cultures

1. The first step in establishing cells in culture is to dissociate organs (e.g. kidney or liver) or tissues into a single cell suspension. It is done by mechanical or enzymatic methods.

2. The cells are transferred into special glass or plastic containers containing culture medium. Under these conditions, maintenance of growth of such cells is called primary cell culture.

3. The cells are enumerated together by a proteinaceous material. Therefore, crude preparation of proteo-lytic enzymes (trypsin and collagenase) are commonly used to break the cementing mater and separate cells of a given tissue. The characteristics of the animal cells govern the characteristics of the cells in culture.

The growing cells are of two different types: (i) anchorage-dependent (adhere cells and (ii) anchorage-independent cells (suspension culture). Commonly the adherent cells can be obtained from such organs that are fixed at a place (e.g. kidney, liver, etc.). The cells are too mobile but remain fixed in connective tissues.

4. In contrast the suspension cells grow continuously in liquid medium and do not attach to the surface of the container. The source of cells is the governing factor for suspension non-adherent cells. All suspension cultures are raised by culturing the blood cells. You know that all blood cells are vascular in nature and get dissolved in plasma.

Secondary Cell Cultures and Cell Lines -

5. The primary cell culture cannot remain viable for a long time because the cell utilise all nutrients of the medium. Therefore, sub- culturing needs to be done on another fresh medium.

6. Hence, the primary culture is removed; adherent cells are dissociated enzymatic ally or by repeated pipe ting. Then the cells are diluted with fresh medium and passed into fresh

culture flask. These sub-cultures are called secondary cell culture or cell lines.

Sub-culturing is done on fresh medium at certain intervals. It provides sufficient nutrient and space to growing cell lines. Characteristic features of such cell type govern that how

quickly sub-culturing shall be done. However, the cells may exhaust the medium and die if they are not split frequently.

During sub-culturing the in vitro conditions produce mostly undefined selection pressure. Consequently, a certain cell type (e.g. fibroblast) is selected. After some generations the normal diploid cells die. Growth of fibroblast cells in culture.

Sometimes some cells of secondary cell cultures in vitro can be transformed spontaneously or chemically. Such transformed cells are immortal (i.e. they will not die) and hence give rise to continuous cell lines i.e. cancerous cell lines.

7. These cells proliferate indefinitely with a doubling time of about 10 to 25 hours. Such cultures consist of a mixture of cell types where a particular cell type may dominate over the others.

APPLICATIONS

A.PLANT TISSUE CULTURE

Plant tissue culture is used widely in the plant sciences, forestry, and in horticulture. Applications include:

- The commercial production of plants used as potting, landscape, and florist subjects, which uses meristem and shoot culture to produce large numbers of identical individuals.
- To conserve rare or endangered plant species.
- A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance.
- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals.
- To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.
- To rapidly study the molecular basis for physiological, biochemical, and reproductive mechanisms in plants, for example in vitro selection for stress tolerant plants.

- To cross-pollinate distantly related species and then tissue culture the resulting embryo which would otherwise normally die (Embryo Rescue).
-
- For chromosome doubling and induction of polyploidy, for example doubled haploids, tetraploids, and other forms of polyploids. This is usually achieved by application of antimitotic agents such as colchicine or oryzalin.
- As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants.
- Certain techniques such as meristem tip culture can be used to produce clean plant material from virused stock, such as potatoes and many species of soft fruit.
- Production of identical sterile hybrid species can be obtained.

B.ANIMAL CELL CULTURE

A. Model Systems-

Cell cultures provide a good model system for studying;

a. Basic cell biology and biochemistry.

b. The interactions between disease-causing agents and cells.

c. The effects of drugs on cells.

d. The process and triggers for aging.

f. Nutritional studies.

B. Toxicity Testing-

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. Especially important are liver and kidney derived cell cultures.

C. Cancer Research-

Since both normal cells and cancer cells can be grown in culture, the basic differences between 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, the mechanisms that cause the change can be studied. Cultured cancer cells also serve as a test system

to determine suitable drugs and methods for selectively destroying types of cancer.

D. Virology-

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. 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.

E. Cell-Based Manufacturing-

While cultured cells can be used to produce many important products, three areas are generating the most interest.

The first is the large-scale production of viruses for use in vaccine production. These include vaccines for polio, rabies, chicken pox, hepatitis B and measles.

The second is the 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. The third is the use of cells as replacement tissues and organs. Artificial skin for use in treating burns and ulcers is the first commercially available product.

F. Genetic Counselling-

Amniocentesis, a diagnostic technique that enables doctors to remove and culture fetal cells from pregnant women, has given doctors an important tool for the early diagnosis of fetal disorders. These cells can then be examined for

abnormalities in their chromosomes and genes using karyotyping, chromosome painting and other molecular techniques.

G. Genetic Engineering-

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 these genes (new proteins).

These techniques can also be used to produce these new proteins in large quantity in cultured cells for further study. Insect cells are widely used as miniature cells factories to express substantial quantities of proteins that they manufacture after being infected with genetically engineered baculoviruses.

H. Drug Screening and Development-

Cell-based assays have become increasingly important for the pharmaceutical industry, not just for cytotoxicity testing but also for high throughput screening of compounds that may have potential use as drugs. Originally, these cell culture tests were done in 96 well plates, but increasing use is now being made of 384 and 1536 well plates.

I. Gene Therapy:

In modern molecular biology, Gene Therapy is an experimental technique that involves insertion of cloned/altered genes into cells using r-DNA technology to replace defective genes causing genetic abnormalities or to prevent potential disorders.

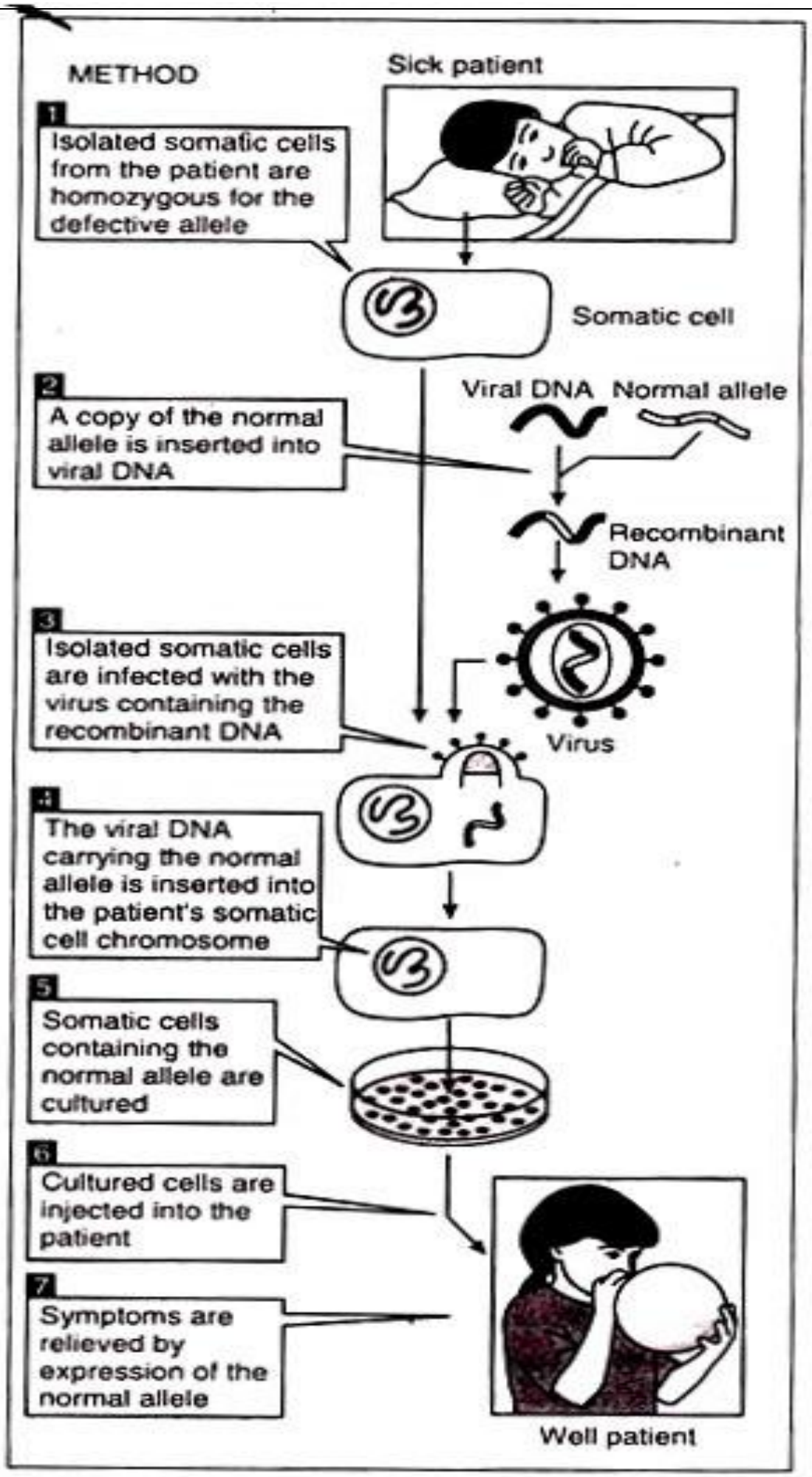


Fig. 13.5: The Principle behind Gene Therapy

ADVANTAGES

A.PLANT TISSUE CULTURE

1. To produce many copies of the same plants then which may be used to produce plants with better flowers, odours, fruits or any other properties of the plants that is beneficial to the human beings.

2. To produce plants anytime we want although the climates are not appropriate to produce a plant. Moreover, if seed is not available, it is possible to produce a plant with this method.

3. If there is plant with partially infected tissue, it is possible to produce a new plant without infection.

4. Very helpful in the genetically modified organism studies.

5.Very useful solution for the prevention of starvation in third world countries since the process is highly efficient, by using only one plant, it is possible to produce more than one thousand of the same plant with higher productive if it's genome changed.

6. The equipments are cheaper when compared to the animal cell culture.

B.ANIMAL CELL CULTURE

1. Control of physicochemical environment- pH, temperature, dissolved gases (O_2 and CO_2), osmolarity.

2. Regulation of physiological conditions-nutrient concentration, cell to cell interactions, hormonal control.

3. The cultured cell lines become homogenous (i.e. cells are identical) after one or two subcultures. This is in contrast to the heterogeneous cells of tissue samples. The homogenous cells are highly useful for a wide range of purposes.

4. It is easy to characterize cells for cytological and immunological studies.

5. Cultured cells can be stored in liquid nitrogen for several years.

6. Due to direct access and contact to the cells, biological studies can be carried out more conveniently. The main advantage is the low quantities of the reagents required in contrast to in vivo studies where most of the reagents (more than 90% in some cases) are lost by distribution to various tissues, and excretion.

7. Utility of tissue cultures will drastically reduce the use of animals for various experiments.

CONCLUSION

Tissue culture is one of the most important part of applied biotechnology. In the coming decades, the world's population will increase more and accommodation space, agricultural lands will decrease significantly. Global climate change is also another consideration. Keeping these in mind we have to ensure a peaceful, healthy and hunger free greener world for our next generation. For doing this there is no alternate of plant tissue culture.

BIBLOGRAPHY

.www.biologydiscussion.com

.www.essays24.com

. nusrat2005.blogspot.com

.www.preservearticles.com

.www.microscopemaster.com

THANK YOU

THE END