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CHAPTER 3

ANIMAL CELL CULTURE AND APPLICATIONS

6.3.1. Introduction

Animal cells, just as plant cells, will continue to grow outside the living organism if supplied with the appropriate nutrients and growth conditions. The process of growing of cells under laboratory conditions is called **Cell Culture**. It is carried out ***in vitro*** ('within glass') as opposed to ***in vivo*** ('within the living'). The advantage and limitations of animal cell culture are given in **Table 1**. A homogenous population of cells derived from a single parental cell is called a **clone**. Therefore, all cells within a clonal population are genetically identical. The growth rate of animal cells is relatively slow and usually require 18 to 24 hour to divide. This makes the animal cell culture vulnerable to contamination, as a small number of bacteria would soon outgrow a larger population of animal cells. The animal cell culture became a routine laboratory technique in 1950s after **George Gay** established the first human cell line (HeLa) from cervix cancer that led to several important discoveries in medical sciences. The need for cell culture, especially at large scales, became apparent with the need for viral vaccines.

Table 1. Advantages and limitations of animal cell culture.

Advantages	Disadvantages
• Homogenous genetic population. • Controlled physico-chemical environment. • Easy to add genes (Transfection) or regulate protein levels (RNAi). • Available in adequate numbers to do chemical study. • Easy production of biopharmaceuticals. • No ethical clearance required. • Cost effective screening assays.	• Small size (high sensitive techniques to detect changes). • Scale-up is challenging. • May not represent *in vivo* phenotype/genotype.

Advent of recombinant DNA technologies in the 1970s, enabled the expression of mammalian genes in bacteria. However, soon it was realized that large proteins or glycoproteins of therapeutic value could not be produced in bacteria. This prompted the usage of animal cell lines for large-scale production of therapeutic proteins. The development of hybridoma technology allowed us to produce large quantities of monoclonal antibodies of immense diagnostic and therapeutic values. Further, recent advances in the area of stem cells, tissue engineering and gene therapy are likely to open new treatment regimens.

Before you learn about the details of animal cell culture technology, it is instructive to know some essential features of animal cell growth in culture.

6.3.2. Animal Cell Culture Techniques

Features of animal cell growth in culture

Animal cells can be grown in glass or plastic vessels, in the presence of nutritive media that need to be is periodically replenished. However, depending on the tissue they have been isolated from, they can be grown only for limited generations even in the best nutritive media. There is a mortality associated with all normal animal cells. Another important feature of animal cells is that they divide and fill the surface of the culture vessel and then stop growing. Relate this to what happens in the animal body. The infant animal grows only to adulthood and not any further. Cells comprising tissues and organs such as the liver grow only to a certain size after which they cease to grow. This phenomenon which occurs in the normal body is observed also in cell culture and is termed **"contact inhibition"**. This means that when cells grow and reach the walls of the container (i.e., reach confluency) they stop growing further. Another important feature of cell growth in culture is that their environment is different from that *in vivo*. These differences affect the adherence of cells to culture vessels, their shape and rate of proliferation. It is of interest to know that in culture, cancer cells appear very different from normal cells. Cancer cells loose contact inhibition and pile on each other due to uncontrolled growth and among other features, appear more rounded in shape. Such differences in growth patterns in normal versus cancer cells are utilized by Oncologists (cancer biologists) to determine whether tumors are cancerous or not using **`Colony formation assay'**. Let us now learn about various types of cell cultures and the technology associated with it.

Primary Cell Cultures

Cells are dissociated from the parental tissue (such as kidney, liver) by mechanical or enzymatic methods and maintained in suitable culture medium and vessels. The most frequently used enzymes for separating cells from a given tissue (dispersion) are crude preparations of trypsin and collagenase that cleave the proteinaceous cementing material between cells in a tissue. The maintenance of growth of such cells under laboratory conditions is known as primary cell culture.

The characteristics of cells in culture usually depend on their original source within the animal.

Cells can be grown as **adherent** (anchorage-dependent) or **suspension cultures** (anchorage-independent). Adherent cells are usually derived from tissues of organs such as kidney where they are not mobile and are embedded in connective tissue. They grow adhering to the cell culture vessel. On the other hand, suspension cells do not attach to the surface of the culture vessel. Virtually all suspension cultures are derived from cells of the blood system. This is because, these cells (e.g., lymphocytes) are also suspended in plasma *in vivo*. The drawbacks of primary culture are that they are time consuming and require the use of live animals or fresh tissue. There can be considerable variation from one preparation to another particularly if prepared by different people. These difficulties can be overcome by the use of **secondary cell cultures** or **cell lines**.

Secondary Cell Cultures and Cell Lines

Once the primary culture is subcultured, it is known as secondary culture or cell line. Subculturing or "splitting cells," is required to periodically provide fresh nutrients and growing space for continuously growing cell lines. The frequency of subculture or density of cells to be plated, depends on the characteristics of each cell type. If cells are split too frequently or at too low a density, the line may be lost. If cells are not split frequently enough, the cells may exhaust the medium and die. Sub-culturing involves: removing the growth media, washing the plate, disassociating the adhered cells, usually enzymatically (e.g. with trypsin), although some cells may be removed by repeated pipeting or gentle scraping), and diluting the cell suspension into fresh media. **Rous** and **Jones** were first to introduce proteolytic enzyme trypsin for the subculture of adherent cells. Such cultures may be called **secondary cultures**. Sometimes, certain cells of these secondary cell cultures can spontaneously become altered (transformed) and give rise to continuous cell lines which show immortality, as they can grow indefinitely without dying in culture. These cultures can contain mixed cell types or can consist predominantly of a single cell type.

Types of Cell Lines

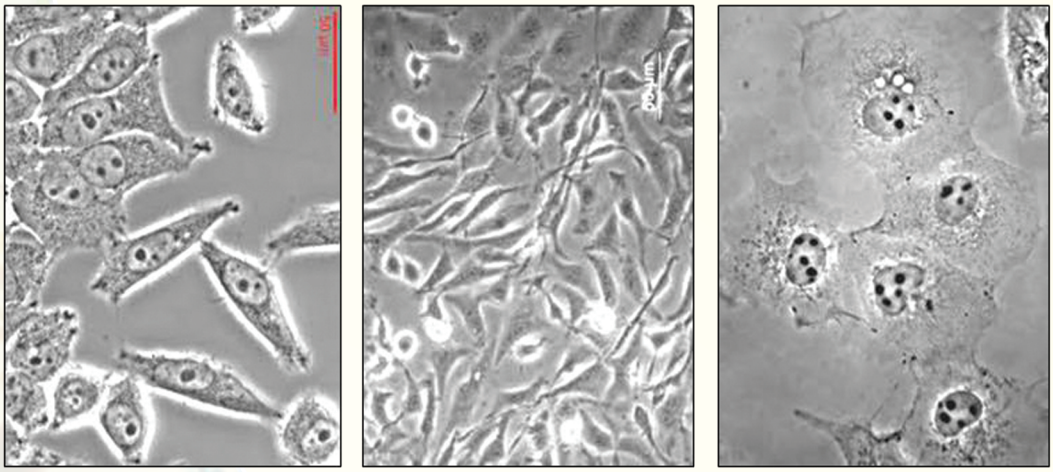
The various types of cell lines are categorized into two types, i.e., finite cell line and continuous cell line.

Finite Cell Lines

Finite cell lines are those cell lines which have a limited life span and they grow through a limited number of cell generations. Finite cell lines show the property of contact inhibition, density limitation and anchorage dependence. The mode of growth is in the monolayer form. The growth rate is slow and doubling time is around 24 to 96 hours.

Continuous Cell Lines

Cell lines transformed under *in vitro* culture conditions give rise to continuous cell lines **(Fig. 1)**. The various properties associated with continuous cell lines are: the **ploidy** (change in basic number of chromosomes), no contact inhibition and no anchorage dependence. The mode of growth is either monolayer or suspension form. The growth rate is rapid and doubling time is between 12 to 24 hours. The density limitation is reduced or lost.



HeLa cell line from human cervical carcinoma

CHO continuous cell line from Chinese hamster ovary

Cos-1 cell line from monkey kidney

Fig. 1. Different mammalian cell lines.

Physical environment for culturing Animal Cells

The culturing of animal cells under *in vitro* condition involves creation of appropriate physical, nutritional and hormonal environments in which the cells can grow. The physical environment includes controlling the temperature, pH, osmolality and gaseous environment by providing a supporting surface and protecting the cells from chemical, physical and mechanical stresses.

Temperature

The mammalian cells are grown in incubators maintained at 37°C. This temperature is chosen because it is the core body temperature of *Homo sapiens*. Further, it has been observed that most cells derived from the warm blooded animals will grow at this temperature.

pH

The regulation of extra-cellular and intra-cellular pH is essential for the survival of mammalian cells. The correct pH is not only important for maintaining the appropriate ion balance but also for maintaining optimal function of cellular enzymes and for optimal binding of hormones and

growth factors to cell surface receptors. Even transient changes in pH can alter cell metabolism which can lead to cell death. Most media strive to achieve and maintain the pH between 7 and 7.4. The regulation of pH is done using a variety of buffering systems. Most media use the bicarbonate - CO_2 buffering system. The interaction of CO_2 derived from cells or atmosphere with water leads to a drop in pH described by the equation below:

$$H_2O + CO_2 \leftrightarrows H_2CO_3 \leftrightarrows H^+ + HCO_3^-$$

The bicarbonate content of the medium neutralizes the effect of increased CO_2 according to the following equation: $NAHCO_3 \rightarrow Na^+ + HCO_3^-$. The increased HCO_3^- ion drives the equation above to the left until the equilibrium is reached at pH 7.4. This kind of system is called an open system.

Osmolality

The osmolality of the culture medium also has a significant bearing on cell growth and function. It preserves the membrane integrity of cells. If the outside osmotic pressure becomes higher or lower than that which must be maintained inside the cell, it will shrink or swell accordingly. The osmolality of the medium used is determined by the media formulation. Salt and glucose are the major contributors to the osmolality of the medium, although amino acids may also contribute significantly. Almost all commercial media are formulated to have a final osmolality of around 300 mOsm. Osmolality can be checked directly with an osmometer.

Medium

The most commonly varied factor in culture systems is the growth medium. Medium is a mixture of inorganic salts and other nutrients capable of sustaining cell survival *in vitro*. Having the correct nutrient mixture can often be the determining factor in failure or success in cell culture. The medium provides essential nutrients that are incorporated into dividing cells, such as, amino acids, fatty acids, sugars, ions, trace elements, vitamins, cofactors, and ions necessary to maintain the proper chemical environment for the cell. Some components may perform both roles; for example, the sodium bicarbonate may be used as a carbonate source but also may play an important role in maintaining the appropriate pH and osmolality. All media contain an energy source, usually glucose. Many of the media contain phenol red as a pH indicator. This is very helpful in monitoring the pH of the culture medium in an incubator. Highly acidic conditions turn the phenol red into yellow while highly alkaline conditions turns the phenol red into pink color.

Serum and antibiotics

Serum is one of the most important components of animal cell culture, as it supports cell proliferation and their attachment to culture vessels. The peptide hormones or hormone-like growth factors that promote healthy growth are often derived from animal blood, such as **foetal**

bovine serum (FBS). Serum is also a source of various amino acids, hormones, lipids, vitamins, polyamines and salts containing ions such as calcium, chloride, ferrous, ferric, potassium etc. Current practice is to minimize using blood-based supplements and switch to **serum-free medium** due to some complications of FBS usage. Although not required for cell growth, **antibiotics** such as penicillin and streptomycin are often used in culture medium to control the growth of bacterial and fungal contaminants.

Vessels and Equipments required for Animal Cell Culture

Cultures should be examined daily for their morphology, colour of the medium and density of cells. The animal cells are usually grown and maintained in Petri dishes, Culture flasks or Multi-well plates of various shapes and sizes **(Fig. 2)** at an appropriate temperature and gas mixture (typically, 37°C, 5% CO_2 for mammalian cells) in an 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.

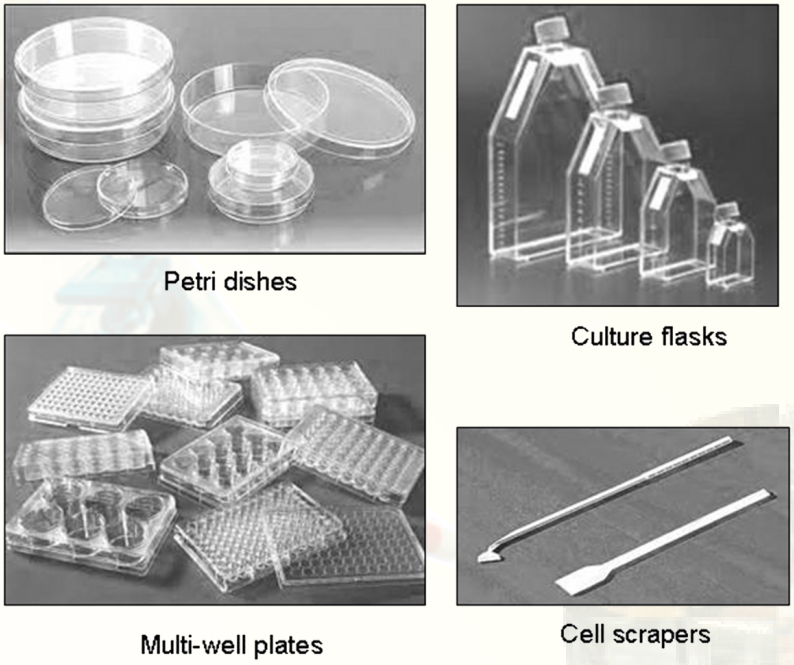


Fig. 2. Vessels and accessories for animal cell culture.

A cell culture laboratory should have equipments like tissue culture hood, CO_2 incubator, inverted microscope, Centrifuge etc. for doing animal cell culture work.

Tissue Culture Hood

All tissue/cell culture manipulations must be performed asceptically, i.e., without any bacterial or fungal contamination. Otherwise, animal cell culture media can easily get contaminated with

bacteria or fungi which will outgrow animal cells. The Laminar Air Flow (LAF) hoods allow the work area to be free of such contamination. A LAF hood essentially performs two functions:

1. Protects the tissue culture from the operator (by providing a sterile environment).
2. Protects the operator from the tissue culture (from possible infection risk).

Depending on the nature of cells/tissue being handled (especially infective agents), the biology safety cabinets are designated as Class I to class III. The LAF hoods have continuous displacement of air that passes through a high efficiency particle air **(HEPA)** filter that removes particulates from the air. The hoods are equipped with a short-wave UV light source that can be turned on for a few minutes to sterilize the surfaces of the hood just before use.

CO_2 Incubator

The CO_2 incubator is designed to reproduce as closely as possible the environmental conditions of the living cells. The essential functions of the incubator are to maintain, the sterility of the chamber, a constant temperature, an atmosphere with a fixed level of CO_2 and high relative humidity. A pan of water is kept at all times in the incubator chamber to maintain high relative humidity and prevent desiccation of the culture medium and maintain the correct osmolarity **(Fig. 3A)**. The animal cells are grown in an atmosphere of 5-10% CO_2 because the medium used is buffered with sodium bicarbonate/carbonic acid and the pH must be strictly maintained.

A. CO_2 Incubator B. Centrifuge C. Inverted Microscope

Fig. 3. Equipments for animal cell culture.

Centrifuge

For most cell culture only low-speed centrifuges are required **(Fig. 3B)**. A gentle braking action helps prevent disruption of the separated bands of cells. In most cases cells should be centrifuged at 20°C; nevertheless low operation temperature is useful to avoid exposing cells to uncontrolled higher temperatures.

Inverted Microscope

In tissue culture vessels, for example a petri dish, the cells are present at the bottom with the culture medium above. The inverted microscope allows the cells at the bottom to be visualized because the optical system is at the bottom with the light source on top **(Fig. 3C)**. Observation of cultures in this way will give an immediate idea of the health and growth of cells. Microscopes should be kept covered to protect from dust and the lights turned down when not in use.

6.3.3. Characterization of Cell Lines

In order to analyze the growth characteristics of a particular cell type or cell line, a growth curve can be established from which one can obtain a population doubling time, a lag time, and a saturation density. A growth curve generally will show the cell population's lag phase, that is, the time it takes for the cells to recover from subculture, attach, and spread; the log phase, in which the cell number begins to increase exponentially and a plateau phase, in which the growth rate slows or stops due to depletion of growth factors and nutrients. An increase in cell number is also a frequently used method of assessing the effect of hormones, nutrients, and so forth on a specific cell type. The culture doubling time, allows prediction of the likely cell concentration at any time in the future **(Fig. 4)**.

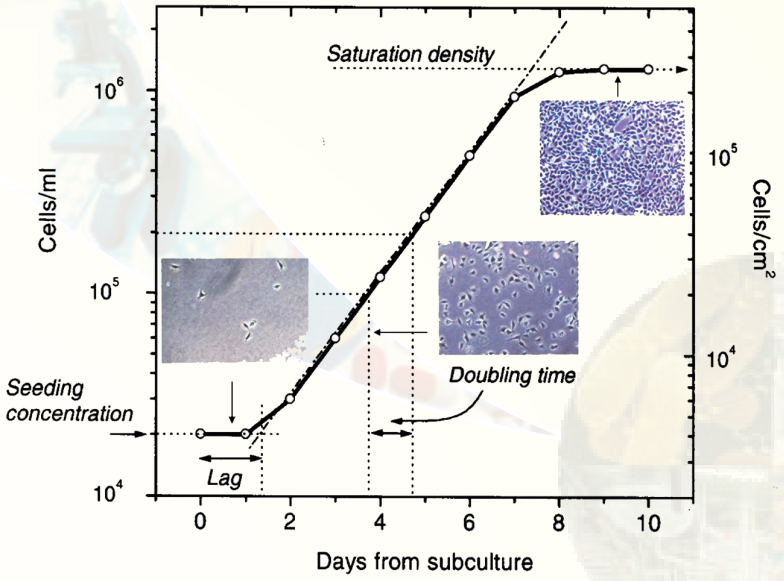


Fig. 4. Normal growth curve of animal cells.

There are laboratories and institutions which maintain various cell lines for scientists to use (for example American Type Culture Collection or ATCC, Virginia, USA). The advantage of using established cell lines is that their growth characteristics, media requirements and responses to selected reagents are established and therefore, convenient for the scientist to use. However, it

is important to check the identity of any newly acquired cell line for species of origin, tissue of origin and the maintenance of specific properties.

Once a cell line has been established, its karyotype has to be determined. This will confirm the species of origin, at least for those cells karyotyped, and determine the extent of gross chromosomal changes in the line. Karyotypes may vary from being near normal (i.e., the vast majority of cells in the culture have normal karyotypes) to being aneuploid. While a normal karyotype is desirable, the presence of an abnormal karyotype does not preclude using the cells for *in vitro* studies, especially if it has been demonstrated that the cells retain the normal function expected of them. If a normal karyotype is required (e.g., cell lines to be used to create transgenic animals), then special care must be taken in handling the cells to minimize chromosomal changes. The stability of the karyotype depends on the species from which the cell lines was derived, the growth conditions used, the way in which the cells are sub-cultured and whether or not the cells are frozen.

Storage and revival of cells

Liquid nitrogen is used for storing cells at very low temperature (-180°C to -196°C). Cells are stored at liquid nitrogen temperatures because the growth of ice crystals is retarded below -130°C. Freezing can be lethal to cells due to the effects of damage by ice crystals, alterations in electrolyte concentration, dehydration and changes in pH. To minimize the effects of freezing, a **cryoprotective agent** such as glycerol or DMSO, is added. A typical freezing medium is 90% serum, 10% DMSO. Further, it is desirable to use healthy cells that are growing in log phase and to replace the medium 24 hours before freezing. Also, the cells should be slowly cooled from room temperature to -80°C to allow the water to move out of the cells before it freezes.

Revival

Frozen cells should be thawed as rapidly as possible by placing the vial containing them into a 37°C water bath with moderate shaking. This is to minimize ice crystal formation, which may damage cells. After thawing the cells can be transferred directly into a tissue culture vessel containing suitable media for further growth.

6.3.4. Methods of Gene Delivery into Cells

A number of methods have been developed over the years for efficient transfer of genes in cell culture. Some common methods of plasmid **DNA transfection** are outlined below:

Calcium phosphate

Here HEPES-buffered saline solution is mixed with a calcium chloride solution containing DNA for transfection to form a fine precipitate of calcium phosphate with DNA. The suspension of the precipitate is then added to the monolayer of cells. The cells take up the calcium-phosphate- DNA complexes by endocytosis and express genes.

Lipofection

In this case, gene is transferred with the help of tiny vesicles of bipolar phospholipids that fuse with the cell membrane, releasing the DNA into the cytoplasm.

Microinjection

It is the most efficient method of gene delivery into cells. Here, DNA is directly injected into the nucleus using a fine glass capillary under a microscope. However this method acquire a great effort as each and every cell has to be injected individually.

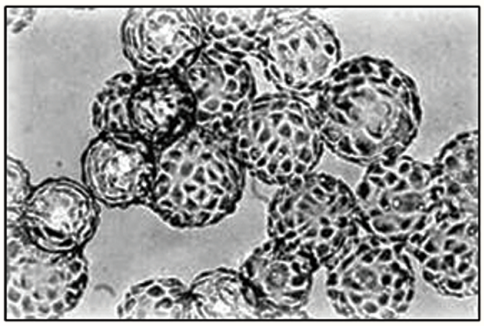
Electroporation

In this method, cells are mixed with the DNA and placed in a small chamber with electrodes connected to a specialized power supply. A brief electric pulse is applied, which is thought to 'punch holes' in the cell membrane, enabling the cell to take up DNA.

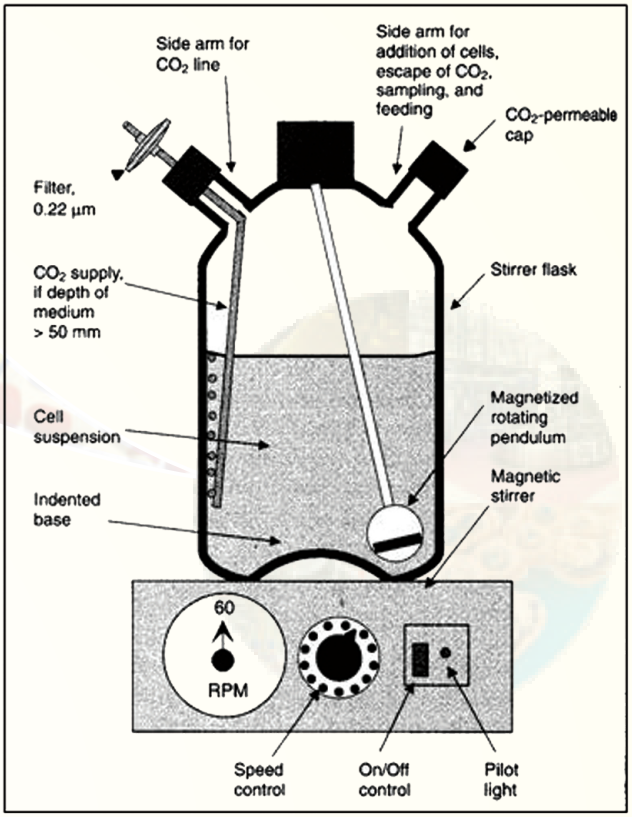
6.3.5. Scale-up of Animal Culture Process

The various scale-up methods include roller bottles with micro carrier beads for adherent cell cultures and spinner flasks for suspension cultures **(Fig. 5).**

A. Roller bottle



B. Microcarrier beads



C. Spinner cell culture

Fig. 5. Tools for scale-up of animal culture process.

Roller bottles

In roller bottles, the cells adhere to the total curved surface area of the micro carrier beads, thereby markedly increasing the available space for growth. These tissue culture bottles can be used in specialized CO_2 incubators with attachments that rotate the bottles along the long axis. After each complete rotation of the bottle, the entire cell monolayer has transiently been exposed to the medium. The volume of medium need only be sufficient to provide a shallow covering over the monolayer **(Fig. 5A)**.

Micro carrier beads

These beads are used to increase the number of adherent cells per flask and are either dextran or glass-based and come in a range of densities and sizes. The beads are buoyant and therefore, can be used with spinner culture flasks. The surface area available for cell growth on these beads is huge **(Fig. 5B)**. Microcarrier beads when re-suspended at the recommended concentration provide an area of 0.24 m^2 for every 100 ml of culture flasks. Under these conditions, adherent cells can be grown to very high densities before crowding becomes a problem. Cells growing at such high densities will rapidly exhaust the medium, which may need replacing the medium during culture.

Spinner cultures

Spinner cultures are used for scaling up the production of suspension cells. They consist of a flat surface glass flask with a suspended central teflon paddle that turns and agitates the medium when placed on a magnetic stirrer. Commercial versions incorporate one or more side arms for sampling and/or decantation. The cells are not allowed to settle to the bottom of the flask and thus cell crowding occurs only at very high densities. Stirring the medium improves gas exchange **(Fig. 5C)**.

6.3.6. Applications of Animal Cell culture

Several medically important protein pharmacueticals have been produced using animal cell culture and recombinant DNA technology. The important ones are listed in **Table 2.**

Table:2

Proteins	Animal Cell Line used	Therapeutic use
Erythropoietin (EPO)	CHO cells	Anemia
Factor VIII	CHO cells	Hemophilia A
Factor IX	CHO cells	Hemophilia B
Follicle Stimulating Hormone (FSH)	CHO cells	Infertility
Human Growth Hormone (hGH)	CHO cells	GH deficiency
Interleukin 2 (IL2)	CHO cells	Cancer therapy
Tissue Plasminogen Activator (t-PA)	CHO cells	Stroke
Monoclonal antibodies (mAbs)	Hybridoma cells	Cancer therapy & Autoimmune diseases

Erythropoietin

Erythropoietin (EPO) is a glycoprotein hormone that is involved in RBC production (erythropoiesis) and wound healing. EPO stimulates the bone marrow to produce more red cells and thereby increase the oxygen-carrying capacity of the blood. It is produced in response to hypoxia (shortage of oxygen) or anoxia (lack of oxygen) caused by anaemia. EPO is useful in the treatment of certain types of anemia such as anemia due to cancer, chronic renal failure and treatment of AIDS. Recombinant human EPO (r-HuEPO) has been produced using Chinese Hamster Ovary (CHO) cell lines. The use of r-HuEPO is advantageous over blood transfusion as it does not require donors or transfusion facilities, and there is no risk of transfusion-associated disease.

Factor VIII

Haemophilia A is a common heritable genetic disorder where the body lacks the ability to produce Factor VIII required for blood clotting. Like EPO, factor VIII is also a glycoprotein and has been produced in CHO cells due to its large structure.

Factor IX

Hemophilia B or Christmas disease is the second most common type of bleeding disorder due to deficiency of factor IX. Recombinant Factor IX produced in CHO cells is used to treat haemophilia B.

Tissue Plasminogen Activator (tPA)

tPA is a serine protease that catalyzes the conversion of plasminogen to plasmin which is responsible for dissolving blood clots. It is approved for use in certain patients having a heart attack or stroke. tPA is the first drug to be produced through mammalian cell culture **(Fig. 6)**.

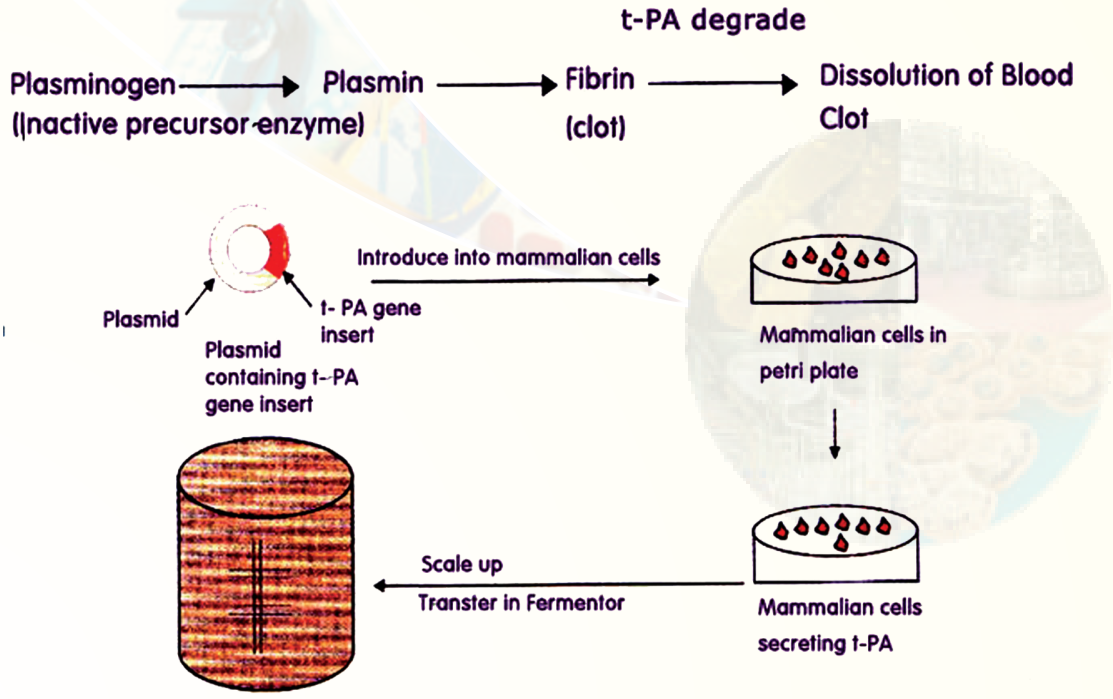


Fig. 6. Production and mode of action of tPA.

Hybridoma Technology for Monoclonal Antibody Production

It has been observed that antibodies bind to specific domains of macromolecules **(antigens)** known as **epitopes**. Antibodies present in serum are a heterologous population released by different populations of B-lymphocytes and therefore are known as polyclonal antibodies. Monoclonal antibodies (mAbs), on the other hand bind specifically to an epitope on an antigen and therefore are useful in detecting specific antigens **(diagnostics)** or blocking their binding by other molecules. mAbs are produced by antigen-activated B lymphocytes that have been immortalised by hybridising (fusing) them with a myeloma cell (cancerous lymphocyte). **Ceasar Milstein** and **George Kohler** (Nobel Prize winners) developed **hybridoma technology** by fusing antibody producing B cells with myeloma cells using polyethylene glycol. The hybrid cells retain the ability of B cells to secrete antibody and the ability of myeloma cells to grow indefinitely. The hybrid clones when grown in culture produces epitope-specific mAb **(Fig. 7)**. This technology has revolutionised the area of diagnostics and antibody-based therapies. The availability of monoclonal antibodies has helped in early detection of many infectious diseases like hepatitis and AIDS.

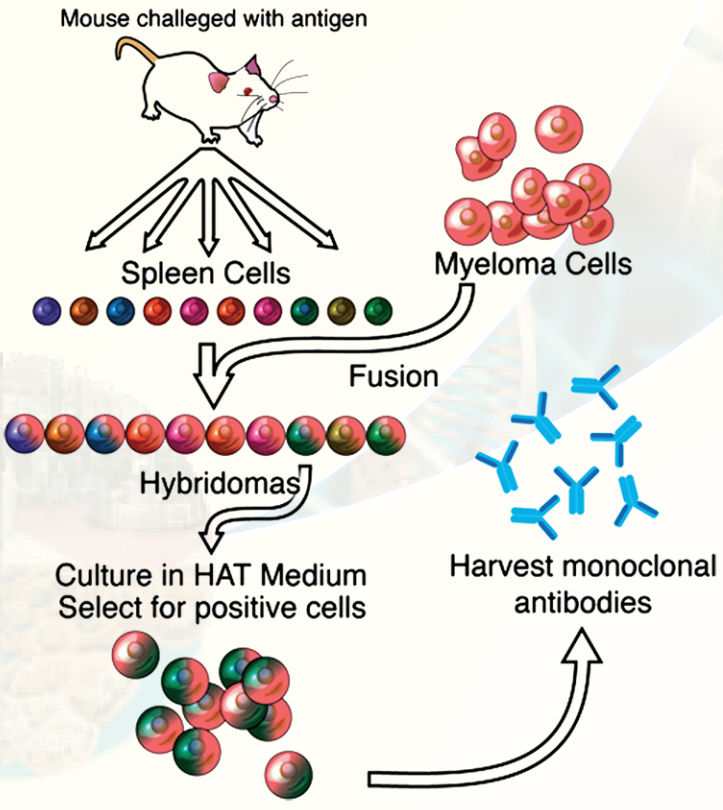


Fig. 7. Production of monoclonal antibodies.

Therapeutic mAb - OKT3

OKT-3 is monab-CD3, an immunosuppressant drug given intravenously to reverse acute rejection of transplanted organs such as heart, kidney and liver. OKT3 is the first monoclonal antibody to be used for the treatment of patients. OKT3 acts by blocking the function of T cells which play a major role in acute graft rejection **(Fig. 8)**. OKT3 binds and blocks the function of a cell surface molecule called CD3 in T cells. The binding of OKT3 to T cells results is followed by blocking of their functions. After OKT3 therapy is over, T cell function usually returns to normal within a week.

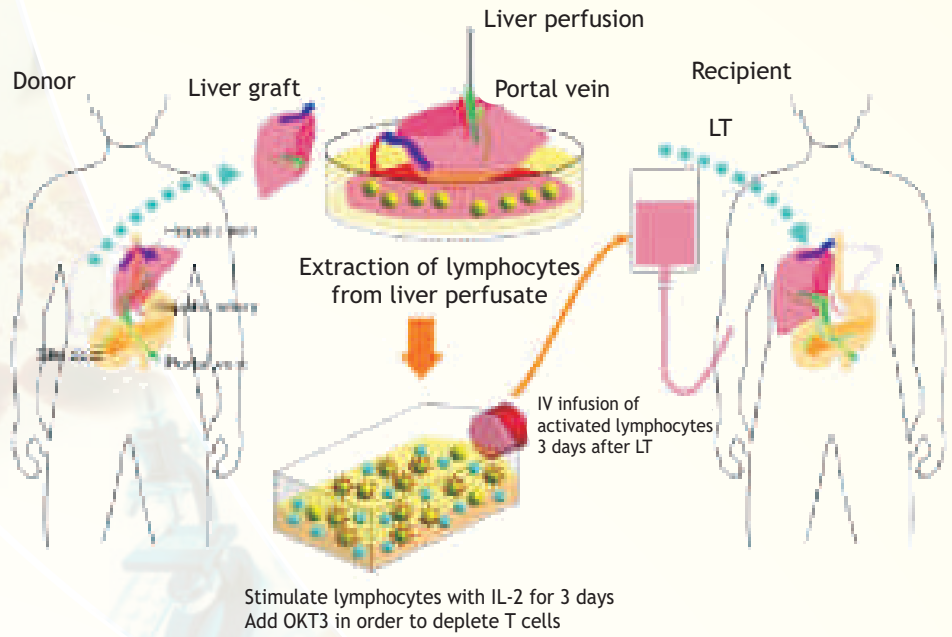


Fig. 8. Adoptive immunotherapy with OKT3 during liver transplantation (LT).

Therapeutic mAb - Herceptin

Herceptin (trastuzumab) is a monoclonal antibody approved for therapy of early-stage breast cancer that is Human Epidermal growth factor Receptor 2-positive (HER2+). These cell surface receptors receive signals that help cells to grow and multiply. Herceptin works by attaching itself to HER2 receptors by blocking them from receiving growth signals. The result is impaired growth of breast cancer.

6.3.7. Stem Cell Technology

Stem cells are characterized by their ability to renew themselves through mitotic cell division and differentiate into a diverse range of specialized cell types. Stem cells are found in all multi cellular organisms. Stem cells are like good shares in the stock market which can either be multiplied **(self renewal)** by getting bonus shares or sold to buy goods **(differentiate)**. Tissues like skin, blood and intestinal epithelium are subject to continuous renewal throughout life and

must maintain an adequate number of cells (stem cells) that retain the potential to proliferate to make good such losses. The most well studied process has been the formation of blood cells (haematopoiesis). It was known in case of mouse that haematopoiesis occurs in the spleen and bone marrow. In human being about 100,000 haematopoietic stem cells produce one billion RBC, one billion platelets, one million T cells, one million B cells per Kg body weight per day. The field of stem cell research was established in 1960s by **Ernest McCulloch** and **James Till** at the University of Toronto.

The two broad types of mammalian stem cells are: **embryonic stem (ES) cells** that are isolated from the inner cell mass of blastocysts, and **adult stem cells** that are found in adult tissues. The ES cells are **pleuripotent** and can differentiate into all types of specialized tissues **(Fig. 9)**. The adult stem cells are **multipotent** (lineage restricted) and act as a repair system for the body by maintaining the normal turnover of regenerative organs, such as, blood, skin, or intestinal tissues **(Fig. 10)**. Stem cells are now routinely grown and transformed into specialized cells such as, muscles or nerves through cell culture and used in medical therapies. The stem cells are useful in many medical conditions where cells are either dead or injured or abnormal, such as:

- Leukemia (cancerous blood cells).
- Heart disease, heart attack (cardiac tissue damage).
- Paralysis (spinal cord injury).
- Alzheimer's, Parkinson's, Huntington's (dead brain cells).
- Burns (damaged skin cells).

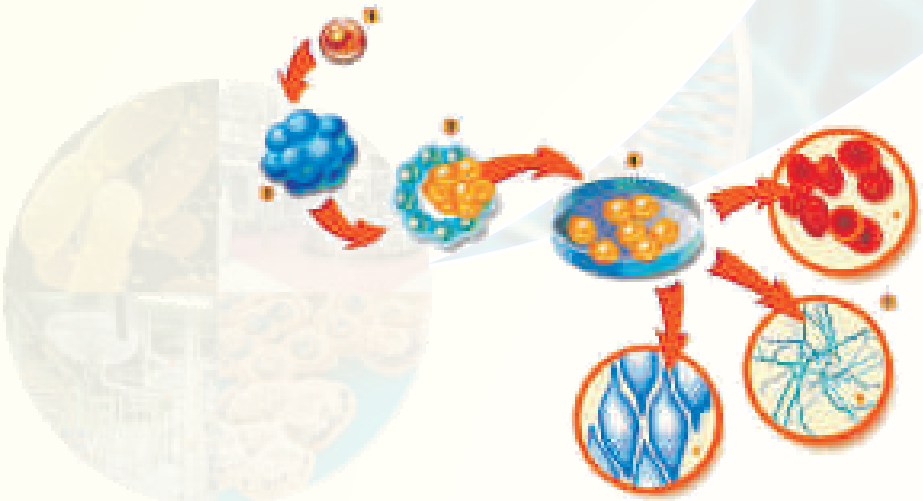


Fig. 9. Cultivation of Embryonic Stem cells. 1, *In vitro* fertilized eggs; 2, Morula; 3, Blastula with inner stem cell mass; 4, Cultured undifferentiated stem cells; 5, Differentiated cells - (a) blood, (b) neural, and (c) muscle cells

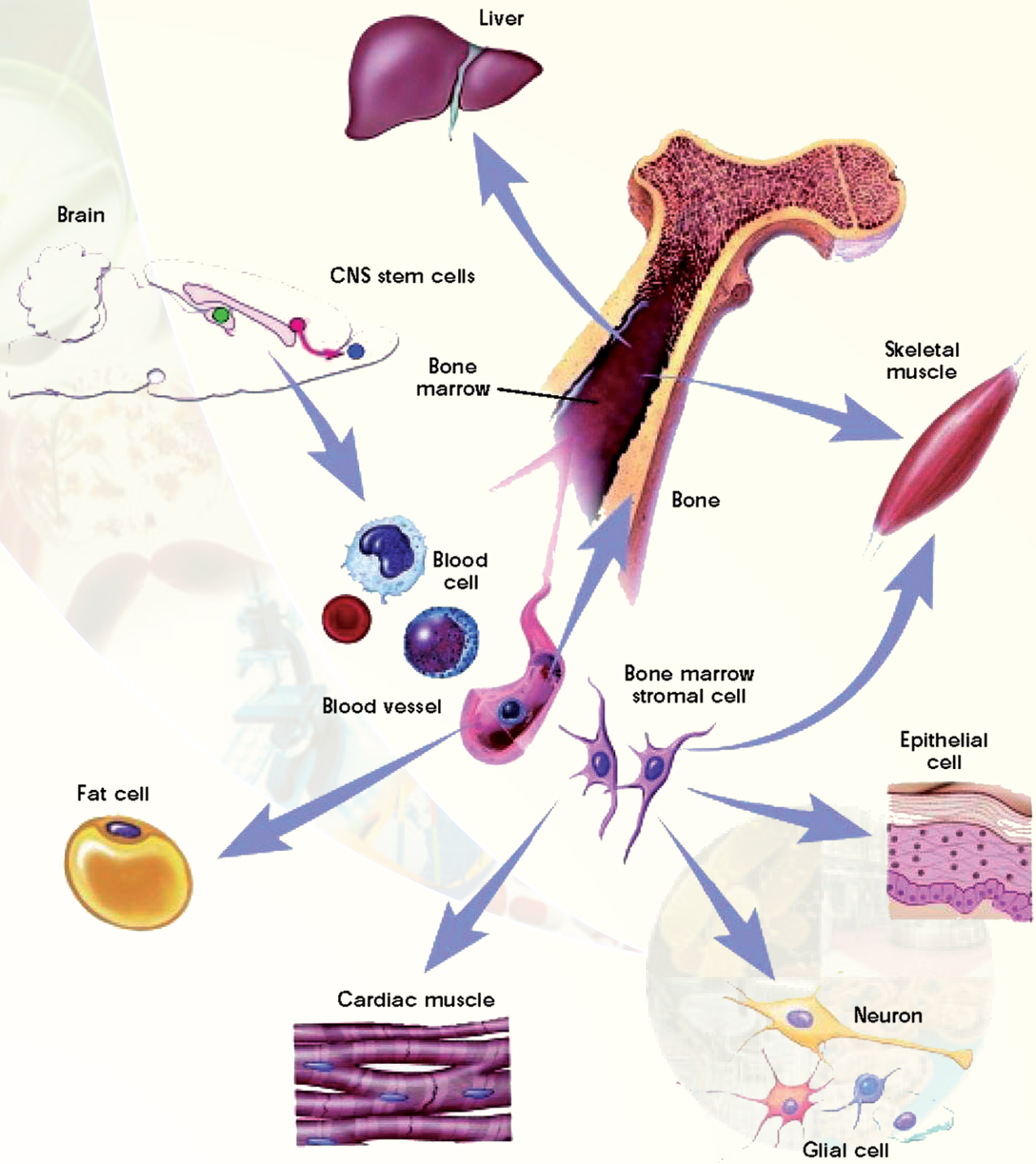


Fig. 10. Cultivation of adult stem cells from bone marrow and their differentiation into specialized cells.

ES Cell culture and its applications

The ES cells are cell lines derived directly from the inner cell mass of growing embryos without use of immortalising or transforming agents. The **inner cell mass** (ICM) of embryos can be maintained in cell culture in the presence of irradiated fibroblast cells. The stem cells:

a. retain the characteristics of founder cells, even after prolonged culture and extensive manipulation.

b. reintegrate fully into embryogenesis if transferred.

c. could be used to create chimeric mice by taking ES cells from a black mouse and implant it into the embryo of an albino mouse (white). The progeny so developed had skin color of black and white (a chimera **Fig. 11**).

d. could maintain a stable euploid karyotype.

e. could self renew without differentiating in culture.

Now it is possible to selectively remove a gene **(gene knock outs)** and make other precise genetic modifications in the mouse ES cells and create mouse models of human diseases. Such mouse models have been extremely useful not only in understanding the genetic basis of a disease but also in search for new diagnostic and therapeutic modalities.

In 1998, **James Thomson** developed a technique to isolate and grow human ES cells in culture. The human ES cells can be derived from the inner cell mass of blastocyst or from human germ cells before they initiate meiosis and cultured in a petri dish. Specialised cells can be grown in the presence of specific growth factors such as fibroblast growth factor and platelet-derived growth factor. The human ES cells have opened new possibilities for stem cell therapy in clinics.

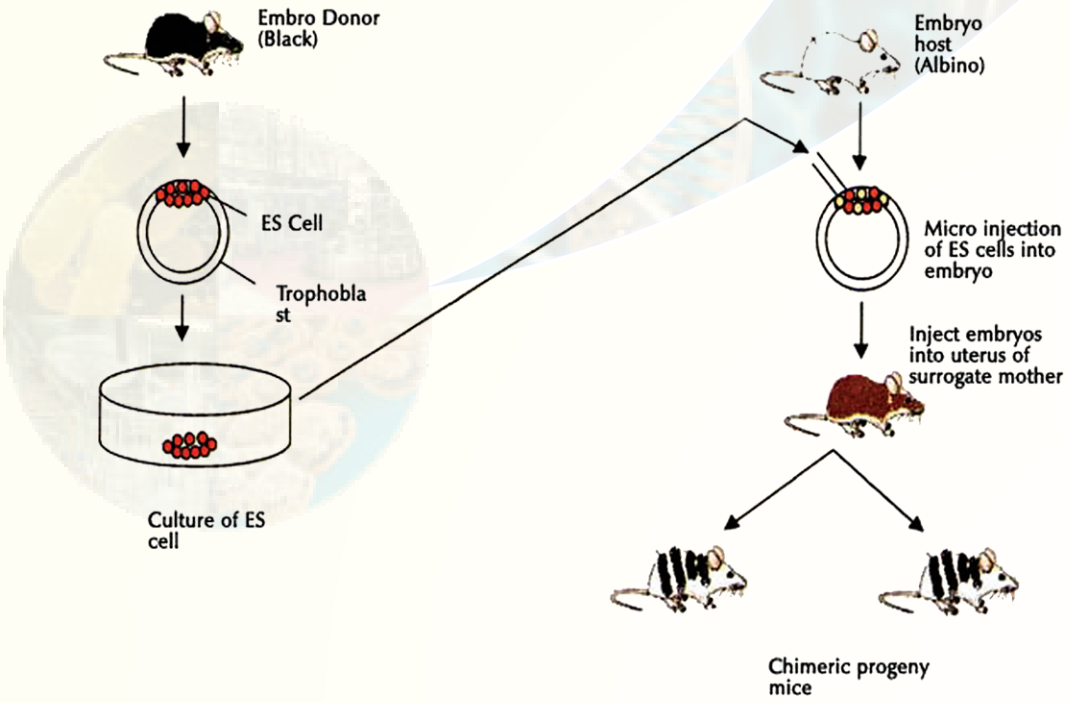


Fig. 11. Creation of chimeric mice using ES cells

6.3.8. Tissue engineering

Recent advances in the fields of cell biology, biomedical engineering and materials science have given rise to the inter-disciplinary field of tissue engineering. The aim of tissue engineering is to supply body parts for repair of damaged tissue and organs, without causing an immune response or infection or mutilating other parts of the body. Tissue engineering potentially offers dramatic improvements in low-cost medical care for hundreds of thousands of patients annually. Large-scale culturing of human or animal cells-including skin, muscle, cartilage, bone, marrow, endothelial and stem cells-may provide substitutes to replace damaged components in humans. Naturally derived or synthetic materials may be engineered into "scaffolds" that when implanted in the body could provide a template that allows the body's own cells to grow and form new tissues **(Fig. 12)**. Such implants could function like neo-organs in patients without triggering immune responses. **Genetically-modified animals** may also provide a source of cells, tissues, and organs for xenografts.

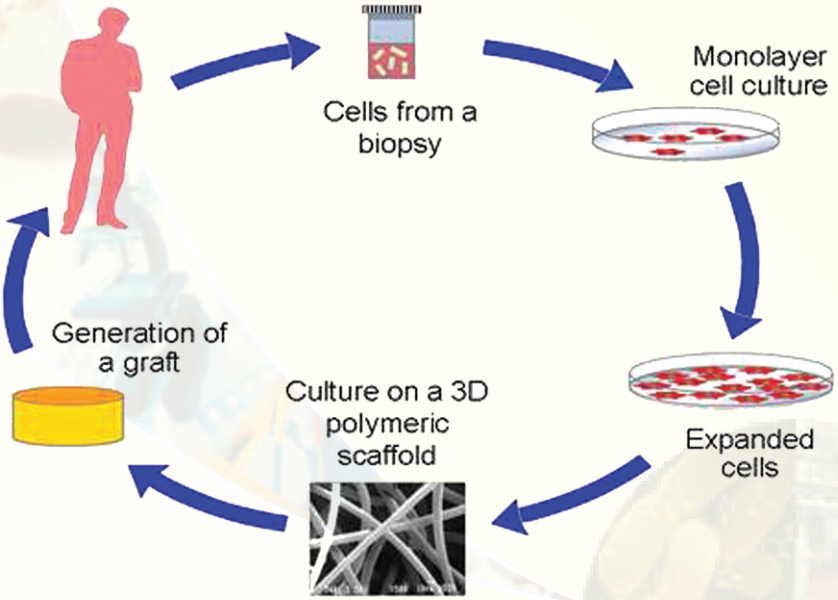


Fig. 12. Basic principle of Tissue engineering.

Review Questions

1. Name two important products from Animal Cell Culture Technology. What are their functions?
2. Write two important features of cultured animal cells. Differentiate between primary and secondary cell cultures.
3. What are cell lines? How the growth characteristics of cell lines determined?

4. What is the importance of pH while culturing animal cells? How is the pH maintained in culture media?
5. What are some of the characteristic features of normal and transformed cells?
6. What is the role of serum for culturing animal cells?
7. Why are $C0_2$ incubators required for animal cell culture?
8. Differentiate between roller bottle and spinner cultures?
9. How are animal cells cryopreserved? Give two examples of cryopreservatives.
10. How is erythropoietin produced by animal cell culture? Write down the procedure involved.
11. What is the mode of action of tPA? How is it produced by animal cell culture technology?
12. How are monoclonal antibodies different from polyclonal antibodies? Write one therapeutic application of monoclonal antibody.
13. What are stem cells? Describe the application of embryonic stem cell technology.
14. What is gene knock out? How is this useful in generating genetic models of human disease?
15. What is meant by tissue engineering? Discuss some important medical applications of tissue engineering.

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