BTY 301 CELL CULTURE TECHNOLOGY & TISSUE ENGINEERING

UNIT – 1

Plant Tissue Culture Media

Tissue culture could be defined as 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. It is also sometimes referred to as 'sterile culture' or 'in vitro culture'. The culture media is provided with water, minerals, vitamins, hormones, carbon sources, and certain antibiotics depending upon the plant being cultured. It should be noted that most plant cells are totipotent and scientists use this characteristic to manipulate plant cells by genetic engineering to regenerate an entire plant. Tissue culture techniques are used to generate large numbers of genetically identical plants for agricultural applications and also grow rare plants.

Nutritional Requirements of Plant Tissue Culture

The culture media for plant tissue culture consists of various nutritional components to sustain the plant's growth. Different plants do need different media, however, specific media have been devised for specific tissue and organs. Some of the important media are:

- White's Medium
- MS (Murashige and Skoog) Medium
- B5 (Gamborg's) Medium
- LS (Linsmaier and Skoog) Medium

Some of the Organic Nutritional Components are:

- Vitamins like thiamine (B1), Pyridoxin (B6), Nicotinic Acid (B3), etc.
- Antibiotics like Streptomycin, Kanamycin
- Amino Acids like Arginine, Asparagine

Inorganic Nutrients that are added are:

Some of the micronutrients are Manganese (Mn), Iron (Fe), Molybdenum (Mo), Zinc (Zn), Copper (Cu), Boron (B).

Six major macronutrients that are included are Nitrogen (N), Sulphur (S), Phosphorus (P), Potassium (K), Magnesium (Mg), Calcium (Ca)

The Components that are used as Carbon and Energy Sources are:

- Lactose
- Maltose
- Galactose
- Raffinose
- Cellobiose

Growth Hormones:

- Auxins- Generally used to induce cell division
- Cytokinins- Used for modifying apical dominance and shoot differentiation
- Abscisic Acid (ABA)- Used occasionally
- Gibberellins- Used occasionally

Plant Tissue Culture Steps

The following is the general process of plant tissue culture. There are specific steps for the regeneration of a complete plant from an explant cultured on the nutrient medium. These steps are:

1. Selection and Sterilisation of Explant: A suitable explant is chosen and excised from the donor plant and the explant is sterilized using disinfectants.
2. Preparation and Sterilisation of the Culture Media: A suitable culture media is prepared with specific components for the growth of the explant, the culture is then sterilized.
3. Inoculation: The sterilized plant is inoculated on the culture medium under aseptic conditions.
4. Incubation: The cultures are then incubated in the culture room with appropriate conditions of light, temperature, and humidity for successful culturing.
5. Sub-Culturing: Cultured cells are transferred to a fresh nutrient medium to obtain the plantlets.
6. Transfer of Plantlets: After the hardening process (i.e., acclimatization of plantlets to the environment), the plantlets are transferred to the greenhouse or in pots.

Types of Plant Tissue Culture

There are 6 types of plant tissue culture techniques. These are:

- **Seed Culture:** Seeds may be cultured in-vitro to generate fully developed plants. It is one of the best methods of tissue culture for raising sterile seedlings. The seed culture is done to get the different types of explants from aseptically grown plants which help in better maintenance of aseptic tissue.

- **Embryo Culture:** Embryo culture is the sterile isolation and growth of an immature or mature embryo in-vitro to grow a viable plant. In some plants, seed dormancy can also be due to mechanical resistance, chemical inhibitors or structures covering the embryo. Excision of embryos and culturing them in nutrient media help in developing seedlings.
- **Meristem Culture:** The apical meristem of shoots of gymnosperms and angiosperms can be cultured to get disease-free plants. Meristem tips, which are between 0.2-0.5 mm, frequently produce virus-free plants and this method is referred to as meristem-tip culture.

  This method is more successful in the case of herbaceous plants than woody plants. In the case of woody plants, success is obtained when the explant is taken after the dormancy period is over. After the shoot tip proliferation, the rooting is done and then the rooted plantlet is potted.
- **Bud Culture**: Buds contain active meristems in the leaf axils which are capable of growing into a shoot. Single node culture is where each node of the stem is cut and allowed to grow on a nutrient medium to be developed into a shoot tip from the axil which ultimately develops into a new plantlet. In the axillary bud method, the axillary buds are isolated from the leaf axils and developed into shoot tips under little high cytokinin concentration.
- **Callus Culture:** Callus is a more or less unorganized de-differentiated mass of cells arising from any kind of explant under in vitro cultural conditions. The cells in the callus are parenchymatous but may or may not be a homogenous mass of cells.

  The callus tissue from various plant species may be different in structure and growth habits. The callus growth is also dependent on factors like the type of explant and the growth conditions. After callus induction, it can be subcultured regularly with an appropriate new medium for growth and maintenance.
- **Cell Suspension Culture**: The growth of individual cells that have been obtained from any kind of explant tissues or callus refers to as the cell suspension culture. These are initiated by transferring pieces of tissue explant/callus into a liquid medium (without agar) and then placing them on a gyratory shaker to provide both aeration and dispersion of cells. Like callus culture, the cells are also sub-cultured into the new medium.

  Cell suspension cultures may be done in batch or continuous culture systems. In a continuous culture system, the culture is continuously supplied with nutrients by the inflow of fresh medium with

subsequent draining out of used medium but the culture volume is constant. This culture method is mainly used for the synthesis of specific metabolites or biomass production

Application of Plant Tissue Culture

The uses of tissue culture are:

- In-plant biotechnology, the useful product is a plantlet and they are used for many purposes.
- All the cells in callus or suspension plant tissue culture are derived from a single explant by mitotic division.
- Hence, all plantlets regenerated from a callus or suspension culture have the same genotype and constitute a clone. These plantlets are utilized in rapid clonal propagation.
- A genetic variation that is observed amongst plant cells of culture is called somaclonal variation.
- A gene that is transferred into an organism by genetic engineering is known as a transgene and it can be introduced into individual plant cells.
- An organism that contains and expresses a transgene is called a transgenic organism.
- The plantlets can be generated from these cells and give rise to highly valuable transgenic plants.
- Mutagens are added to single-cell liquid cultures for the induction of mutations.
- Tolerance to stress like toxins, salts, drought, pollutants, flooding, etc. can also be obtained by providing them in a culture medium by increasing dosage. The surviving healthy cells are taken to a solid medium for raising resistant plants.

Application of Plant Tissue Culture in Agriculture

Tissue culture has been widely applied for more than half a century and is now used to improve many crops important to developing country food security including major staples such as rice, potato, and banana.

Crops that are important to develop countries that are improved and propagated by tissue culture include:

- Cassava, sweet potato, and yam
- Commercial Plantation Crops: coffee, cocoa, sugar cane, oil palm, and tea
- Horticultural Crops: cardamom, artichoke, garlic, ginger, and vanilla
- Fruit Trees: almond, citrus, coconut, date palm, grape, lemon, olive, pistachio, pineapple, etc.

Some of the countries with well-developed tissue culture programs are Gabon, Kenya, Nigeria, and Uganda.

Some of the greatest successes with tissue culture have been demonstrated with vegetatively propagated root crops. For example, disease-free sweet potatoes have been adopted on 500,000 hectares in Shandong Province in China, increasing yields between 30%–40% and incomes for 7 million sweet potato producers by 3.6%–1.6%.

In India, potato breeders have started using tissue culture to detect viruses at the initial stages of seed production. This has led to an estimated 2 to 3 fold increase in seed health whilst generating more than $4 million in revenues.

Farmers in Vietnam participate in the use of tissue culture for high-yielding, late-blight resistant potatoes. This has enabled them to double their yields from 10 to 20 tonnes per hectare.

Somatic Embryogenesis Definition

Somatic embryogenesis is the process wherein somatic cells differentiate into somatic embryos. It is not a naturally occurring process, an artificial one wherein an embryo or plant is obtained from one somatic cell. Somatic embryos take form from the cells of the plants, which usually do not take part in embryo development. Neither a seed coat nor endosperm is formed around the somatic embryo.

In the process, one cell or a cluster of cells initiates the developmental route, which results in reproducible regeneration of non-zygotic embryos, which can germinate for the formation of an entire plant.

The cells which are derived from potential source tissues are subject to a culture medium for the formation of an undifferentiated cluster of cells referred to as the callus. In the tissue culture medium, the plant growth regulators can be formed for the induction of the formation of calluses and hence modified to induce the embryos for the formation of calluses.

Process of Somatic Embryogenesis

The somatic embryogenesis procedure is a three-step procedure, which causes the induction of embryogenesis, development of the embryo and its maturation.

The principle of somatic embryogenesis finds its basis on the topic of totipotency of the plant cells; it illustrates two facets of plant embryogenesis:

- The process of fertilization can be replaced by an endogenous mechanism.
- The other types of cells of the plant, apart from the fertilized egg cells, can retrieve the capacity to form an embryo.

Since the process of somatic embryogenesis does not entail the procedure of fertilization, it promotes the large scale propagation of plants at a faster rate. In addition, it also assists in the genetic transformation of plants, serving as a promising resource for the cryo-storage of the embryo and germplasm.

Somatic embryogenesis – Induction

Cells are reactivated to differentiate and develop embryos, which occur through two processes: direct somatic embryogenesis and indirect somatic embryogenesis.

Direct somatic embryogenesis

It involves the development of the embryos in a direct way from the cells of the explants, such as the cells of the immature embryos. Here, there is no intermediary stage (like the formation of the callus). The explants of the somatic embryogenesis are seen to entail PEDCs (pre-embryogenic determined cells).

Indirect somatic embryogenesis

It includes the formation of somatic embryos by reiterating numerous cycles of cell divisions. It includes intermediary steps of growth of the callus, and hence the process includes multiple steps.

The cells which do not carry the pre-embryogenic determined cells are caused to differentiate for the formation of the embryo by revealing different treatments. The cells modify into IEDs (induced embryogenic pre-determined cells).

Types of Somatic Embryogenesis

Somatic embryogenesis is of two types:

- Direct somatic embryogenesis

Here, the embryos start directly from the explants when callus formation does not take place. Embryos, in this case, are formed as a result of Pre-induced Embryogenic Determined Cells (PEDCs).

- Indirect somatic embryogenesis

The callus from the explants occurs from where the embryo develops. Here, the embryos are formed as a result of Induced Embryogenic Determined Cells (IEDCs).

Advantages of Somatic Embryogenesis

In comparison with zygotic embryogenesis, somatic embryogenesis has these benefits:

- A huge number of embryos are obtained

- The development and environmental stage of somatic embryos can be regulated
- This process of embryogenesis can be monitored easily

The significance of somatic embryogenesis is as follows:

- Production of artificial seeds
- Higher rate of propagation
- Apt in suspension culture
- Labour savings

Factors Affecting Somatic Embryogenesis

The aspects which affect the process of somatic embryogenesis are as follows:

Traits of explant

Despite the fact that variations of explants can be used, the apt stage of development of explants is vital too to initiate the embryogenic callus; whereas juvenile explants tend to give rise to more somatic embryos compared to older explants. Also, different explant explants tissues from the same mother plant generated embryogenic callus at varying frequencies.

The desired species of plants to be induced for embryogenesis decides the choice of explants. For the majority of plant species, explants of immature zygotic embryos are apt for somatic embryogenesis.

Growth regulators

Cytokinins: These have been in use in the primary medium consistently at the time of embryogenesis of the crop plants. They are vital in speeding up the process of maturation of somatic embryos, the cotyledon development, precisely.

Auxins: These alone or in combination with cytokinin seemingly are vital for the start of growth and the induction of the embryogenesis of all the auxins. Auxins find immense importance in the first step of this process – the step of induction. High levels of auxins can lead to the inhibition of embryogenesis in the explants of the citrus plants.

Abscisic acid: These are supplied at the inhibitory levels. It facilitates the development and maturation of the somatic embryos, while also inhibiting the unusual proliferation and the initiation of the accessory embryos.

Genotype

The process of embryogenesis is also affected by the genotypic variation seen in different plants; as per research, it can also be as a result of the endogenous levels of the hormones.

Sources of nitrogen

Nitrogen forms that are utilised in the media have an influence on the process of embryogenesis in plants. Forms of nitrogen have a marked influence on somatic embryogenesis. Somatic embryo development takes place on a medium that contains NO^{-}_{3} as the only source of nitrogen.

Polyamines

The concentration of polyamines in media or explants is said to have an effect on the process. Experts observe the concentration of polyamines to be seen in higher concentrations in the polyembryonates compared to monoembryonates.

Electrical stimulation

Electrical stimuli apparently facilitate the differentiation of the structured embryo by influencing the cell polarity via modifications in the structure of the microtubules and the induction of first asymmetric division.

Somatic Embryogenesis Stages – Steps of Somatic Embryogenesis

The process of somatic embryogenesis occurs in the following stages:

Induction

For the process of induction, auxins, specifically 2, 4-D are typically essential. The necessity of exogenous auxin to induce somatic embryogenesis is based on the nature of the explants, which are made use of with a proportional concentration of the auxins.

Development

Once reinitiation of the process of cell division and a stage of cell proliferation occurs in the presence of auxins, embryogenic cells are liberated in the auxin-free medium. Such cells are in groups of cytoplasmic cells referred to as the PEMs (Pro Embryonic Mass of Cells).

Maturation

The standard of the somatic embryos in aspects of their conversion into plants or germinability is degraded as a result of usually normal-seeming somatic embryos, which in actuality are incomplete in their development. The somatic embryos, as opposed to seed embryos, do not experience the last stage of embryogenesis referred to as embryo maturation that is distinguished by the collection of embryo-specific

reserve food substances and proteins imparting desiccation tolerance to the embryos. The size of the embryos does not increase at this stage.

Cell culture

Cell culture technology involves growing cells in a controlled environment outside their natural setting. There are several types of cell culture techniques, each suited to different types of cells and research purposes. Here are the main types:

1. Primary Cell Culture

Primary cell culture involves the direct extraction of cells from living tissues and their subsequent growth in culture. These cells have a limited lifespan and closely resemble the tissue of origin in terms of structure and function.

Advantages: More physiologically relevant, less likely to undergo genetic mutations.

Disadvantages: Limited lifespan, potential contamination, more complex to culture.

2. Secondary Cell Culture

Secondary cell culture involves the sub-culturing or passaging of primary cells. This means transferring cells from a primary culture to a new vessel to allow for continued growth.

Advantages: Extended lifespan compared to primary cultures.

Disadvantages: Cells may undergo genetic changes over time, potential for senescence.

3. Cell Lines

Cell lines are cultures of cells that can proliferate indefinitely. They are often derived from cancerous tissues or treated to become immortalized.

Advantages: Unlimited supply of cells, reproducibility, easy to maintain.

Disadvantages: May not accurately represent normal cell behavior, potential genetic instability.

Types of Cell Lines:

Continuous Cell Lines: These are immortalized cells that can divide indefinitely (e.g., HeLa cells).

Finite Cell Lines: These are cells that have a limited number of divisions before they stop proliferating (e.g., WI-38 human fibroblast cells).

4. Organotypic Cultures

Organotypic cultures involve growing cells in a 3D structure that mimics the organization and function of the original tissue.

Advantages: Better mimicry of in vivo conditions, useful for studying tissue architecture and function.

Disadvantages: More complex to set up and maintain, can be more expensive.

5. 3D Cell Cultures

These cultures allow cells to grow in three dimensions, which can better replicate the environment in tissues.

Types:

Spheroid Cultures: Cells aggregate to form spherical structures.

Organoid Cultures: Derived from stem cells, these form miniaturized versions of organs.

Scaffold-based Cultures: Use a 3D scaffold to support cell growth.

Advantages: More accurate representation of in vivo conditions, better for drug testing and cancer research.

Disadvantages: More complex, higher cost, and challenging to maintain.

6. Co-cultures

Co-cultures involve growing two or more different cell types together to study their interactions.

Advantages: Allows study of cell-cell interactions, more physiologically relevant.

Disadvantages: Increased complexity, potential for cross-contamination.

7. Stem Cell Cultures

Cultures of stem cells, which have the ability to differentiate into various cell types.

Advantages: Versatile, can be used to generate different types of cells for research and therapy.

Disadvantages: Require specific conditions to maintain pluripotency, ethical considerations (particularly for embryonic stem cells).

Applications of Cell Culture Technology:

Basic Research: Understanding cell biology, genetics, and biochemistry.

Drug Development and Testing: Screening for efficacy and toxicity.

Regenerative Medicine: Development of tissue-engineered products.

Cancer Research: Studying cancer cell biology and testing potential treatments.

Vaccine Production: Growing viruses for vaccine development.

Toxicology Testing: Assessing the safety of chemicals and products.

Each type of cell culture has its unique advantages and limitations, making them suitable for different research and application purposes.

Protoplast Culture: Isolation and Culture Methods

Introduction

Protoplast is defined as naked plant cells or plant cells without a cell wall. It consists of plasmalemma containing all the other cellular content or components in it. In tissue culture labs it's used to regenerate a whole plant providing suitable artificial medium and environmental conditions. This process is known as protoplast culture.

The protoplast term was first introduced by the scientist Hanstein (1880). And, its first isolation was done by Klercker (1892) using a mechanical method. However, the serious efforts in the field of protoplast culture started in 1960, when a scientist named Cocking isolated the protoplast using enzymatic techniques.

Protoplasts of different species are generally fused to produce hybrid plants. The process is known as **somatic hybridization (or protoplast fusion)**. Often a normal protoplast of the plant is also fused with a protoplast without a nucleus (enucleated protoplast) to form a cybrid or cytoplasmic hybrid. The process is known as **cybridization**.

In protoplast culture, protoplasts isolated from any plant part, including root, shoot, leaves, or embryo, is cultured in an artificial media under artificial conditions favoring cell division and plant regeneration.

This article presents a bring on the isolation techniques of protoplasts and how they are cultured in labs for the regeneration of plants.

Protoplast Isolation and Culture

Protoplast culture involves the following culture events:

- Leaves of the specific plant are taken and sterilized.
- The epidermis layer of the leaf is peeled and the leaf is cut into small segments.
- The leaf segments are put into an enzyme mixture for protoplasts' release.
- Then, the enzyme solution is collected and suspended in a washing tube, which is centrifuged.
- The obtained pellets are resuspended in a sucrose washing medium and centrifuged again.
- The protoplasts are separated at the top of the tube in the form of a band.
- The protoplast bands are suspended in a culture medium.
- The protoplasts are isolated and indued for wall formation.
- After the wall formation, the cells enter into the division phase, forming a clump of a few tissues followed by callus formation.
- Shoots are differentiated in callus and then plantlets are regenerated leading to the forming of a whole plant.

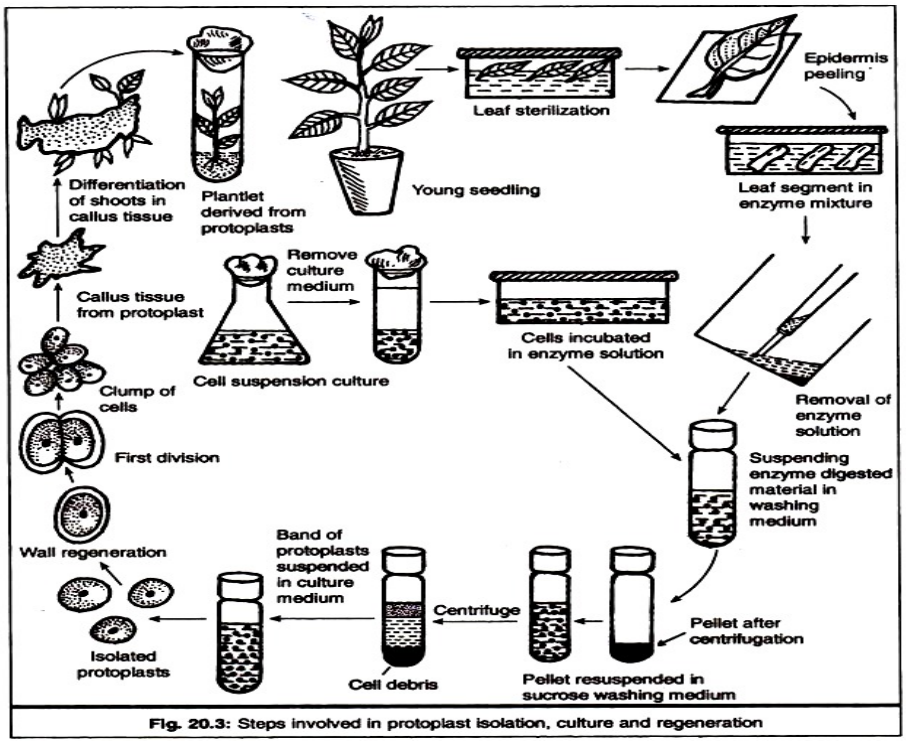

Fig. 20.3: Steps involved in protoplast isolation, culture and regeneration

Protoplast Isolation

Protoplasts can be isolated from different parts of the plants including, roost leaves, stems, microspores, embryos, and fruits. However, among all these sources, the mesophyll tissues of the expanded leaves are the most preferred source of the protoplast isolation.

Protoplasts in labs are isolated mainly using two techniques:

Mechanical Method

In this method, a small piece of the epidermis of the plant is taken and subjected to plasmolysis, causing protoplasts to shrink away from the cell wall. Then the tissue is dissected for the release of the protoplasts.

This method of protoplast isolation is a tedious process and only helps in the isolation of only a few protoplasts. The other limitations are that the viability of the yielded protoplasts is low. However, still, the technique is preferred by some labs because of the deleterious effects of the enzymes used in the enzymatic approach to protoplast isolation.

Enzymatic Method

It's the most widely used technique of protoplast isolation. In this method, protoplasts are isolated from the source using enzymatic solutions. This technique is faster, efficient, and releases more number of viable protoplasts without any damage.

The plant cell wall is composed of cellulose, hemicellulose, and pectin, thus, the enzymes used for protoplast isolation include cellulase, hemicellulase, and pectinase. The incubation period of protoplast sources in enzymes depends on the type of enzymes used to prepare the solution.

There are two ways of isolating protoplasts using the enzymatic technique. It includes:

- **Sequential Method:** It involves the use of two enzymes, pectinase, and cellulase, First, pectinase separates cells from middle lamella, and then cellulase separates the protoplast from the rest of the cell wall.
- **Simultaneous Method:** In this method, both macerozymes and cellulase are used at the same time for complete protoplast isolation.

Protoplast Purification

The separated protoplasts, using mechanical or enzymatic techniques are put under the purification process. Here, all the undigested cells, undigested tissues, and damaged protoplasts are filtered out.

After filtration, the obtained protoplasts are centrifuged, washed, and recovered above Percoll.

Viability of Protoplasts

For successful culturing and increased yield, it's essential to test the viability of the protoplasts. Some of the methods are mentioned below:

- Fluorescein diacetate (FDA) staining method
- Phenosafranine staining method
- Calcofluor white (CFW) staining method
- Oxygen Uptake measurement technique
- Method Involving the Measurement of Protoplasts' Photosynthetic Activity

Protoplast Culture Media Types

The culture medium contains all the nutrients and vitamins required for the growth and development of plants in lab conditions. Mostly MS media is used for such purposes. However, often a modified MS media or B5 media is found to be suitable for the regeneration of plants. It means the formulation of the culture media depends on the species of the plants and their specific growth requirements.

Some special feature of protoplast culture media are given below:

- There should be less iron and zinc and no ammonia in the culture medium.
- For the membrane stability, the calcium concentration should be 2-4 times more compared to that used in normal cell culture media.
- Mainly glucose is the preferred source of carbon, however, a combination of glucose and sucrose can also be used.
- A high auxin/kinetin ratio is required to induce cell divisions, however, a high kinetin/auxin ratio is needed for regeneration.
- Some vitamins can be used as they are used in standard tissue culture media.

Other than the nutritional components, the osmoticum (the chemical which increases the osmotic pressure of the solution) is another factor that needs to be taken care of while preparing the tissue culture media.

For plant regeneration from protoplasts, first, they are required to develop cell walls to proceed to the stage of cell division. Protoplast is cultured either on semi-solid media or liquid media. However, there are also instances where protoplasts are first cultured on liquid media and then transferred to solid media for further development.

Agar Culture

Agar is the most widely used solidifying agent in labs. As much concentration of agar should only be used during the experiments that it forms a soft layer of the gel after being mixed with protoplast suspension.

The protoplasts are cultured on the plate using Bergmann's cell plating technique. Here, the protoplasts are fixed in a position for cell division and further growth. The agar technique is efficient to prevent the formation of clumps in the cultures.

Liquid culture

It's the most preferred method for the protoplast culture. Using this technique, the density of the protoplasts can be manipulated and they are easy to dilute and transfer. Moreover, the osmotic pressure of the cultures can also be diluted.

Protoplast Culture Methods

The protoplasts are cultured in labs using the following techniques:

- Feed Layer Technique In this technique protoplast suspension is exposed to X-rays and then plated on agar plates. It's used to culture protoplasts at low density. Moreover, the feel layer technique is also suitable for the selection of hybrid cells and specific mutants on plates.
- Co-culture Protoplasts This technique is used to culture together protoplasts of two different species. And, you must also note that it can only be used if the protoplasts of the two plant species are morphologically distinct.
- Micro-Drop Culture In this technique, protoplasts are cultures in a special dish, known as Cuprak dishes. The dish has outer and inner chambers. The inner chamber has several walls, in which individual protoplast droplets in nutrient media can be added. The outer chamber contains water to maintain the humidity for the growth of the cultures.

UNIT - 2

Animal Cell & Tissue Culture

Animal cell culture is a type of biotechnological technique where animal cells are artificially grown in a favorable environment**.**

- The cells used in animal cell culture are usually obtained from multicellular eukaryotes and their established cell lines.
- Animal cell culture is a common and widely used technique for the isolation of cells and their culture under artificial conditions.
- This technique was developed as a laboratory technique for particular studies; however, it has since been developed to maintain live cell lines as a separate entity from the original source.
- The development of animal cell culture techniques is due to the development of basic tissue culture media, which enables the working of a wide variety of cells under different conditions.
- In vitro culture of isolated cells from different animals has helped in the discovery of different functions and mechanisms of operations of different cells.
- Some of the areas where animal cell culture has found most applications include cancer research, vaccine production, and gene therapy.
- The growth of animal cells on artificial media is difficult than growing microorganisms on artificial media and thus, require more nutrients and growth factors.
- However, advances in the culture media have made it possible to culture both undifferentiated and differentiated cells on artificial media.
- Animal cell cultures can be performed from different complexities of cells as complex structures like organs can also be used to initiate organ culture in vitro.
- Depending on the purpose and application of the technique, cells, tissues, or organs can be used for the culture process.

Types of Animal cell culture

Animal cell cultures can be divided into two distinct groups depending on the number of cell divisions occurring during the process;

1. Primary cell culture

- Primary cell culture is the first culture obtained directly from animal tissue via mechanical and chemical disintegration or enzymatic methods.
- The cells of the primary cell culture are slow-growing cells that carry all the characteristics of the original tissue or cells.
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- Since these cultures are obtained directly from the origin, they have the same number of chromosomes as the original cells.
- Primary cell cultures are performed in order to preserve and maintain the growth of cells on an artificial growth medium at a particular condition.
- Primary cell cultures can be subcultured to obtained other cultures that either continue to grow indefinitely or die after a few subcultures.
- The subsequent subculture of primary cell culture results in the introduction of mutations into the cells, which might result in cell lines.
- The morphology of cells in the primary cell cultures might be different and varied, with the most commonly observed morphological structures being epithelium type, epithelioid type, fibroblast type, and connective tissue type.
- Primary cell cultures are difficult to obtain and usually have a shorter lifespan. Besides, these are prone to contamination by bacteria and viruses.
- The increase in cell numbers in the primary cell culture can result in exhaustion of the substrate and nutrients, which affects cellular activity.
- Usually, primary cell cultures need to be subcultured in order to maintain continuous cell growth once they reach the confluence stage.
- Primary cell cultures can be further divided into two groups depending on the kind of cells present in the culture;

a. Anchorage-dependent/Adherent cells

- The cells in the culture require a stable biologically inert surface for adherence and growth.
- The surface should be solid and nontoxic as these cells are difficult to grow as cell suspensions.
- These cells are usually obtained from the tissues of organs where the cells remain immobilized within the connective tissue.
- Examples of adherent cells include kidney cells and mouse fibroblast STO cells.

b. Anchorage-independent/ Suspension cells

- These cells can grow efficiently as cell suspensions and do not require a solid surface for attachment.
- These can be grown on liquid media continuously to obtain fresh subcultures.
- The ability of the cells to grow as suspension depends on the source of cells as cells that remain as suspensions in the body are effective suspension cells.
- Examples of suspension cells include blood cells that are vascular and remain suspended in the plasma.

2. Secondary cell culture

- Secondary cell cultures are obtained after the primary cell cultures are subsequently subcultured over a period of time in fresh culture media.
- The cells of the secondary cell cultures are long-lasting as these have a higher lifespan due to the availability of appropriate nutrients at regular intervals of time.
- Secondary cell cultures are favored over primary cell cultures as these are more readily available and are easy to grow and preserve.
- These are formed from the enzymatic treatment of the adherent cells followed by washing and resuspension of cells in particular volumes of fresh media.
- Secondary cell cultures are prepared when the number of cells in the primary culture exceeds the capacity of the medium to support growth.
- Secondary cultures help to maintain an optimal cell density necessary for continued growth.
- The cells of the secondary cell culture might not resemble that on the parental tissue as mutations, and genetic alterations might be introduced during the subculture process.
- The cells can be transformed as, in some cases, the continuous subculture can lead to immortal cells.
- The risk of contamination by bacteria and viruses is less as the cells transform and become less susceptible to infections.
- An important disadvantage associated with secondary cell culture is that the cells might develop the tendency to differentiate over a long period of time and result in aberrant cells.

Procedure of Animal cell culture

1. Growth Conditions

- Animal cell culture requires the use of specific culture media that are more complex and specific than the basic culture media used for microbial growth.
- Some of the important basic components of the media are inorganic salts, nitrogen source, energy source, vitamins, fat and fat-soluble vitamins, growth factors, and hormones. In some cases, pH buffering systems and antibiotics are also added.

- The temperature for the growth depends on the source of the cell as different organisms require different temperatures for cell growth and division.
- Warm-blooded animal cells can be cultured at 37℃ as the optimal temperature, whereas cold-blooded animals grow between 15℃-25℃.

2. Primary cell culture

- Primary cell cultures are obtained from fresh tissues that are removed from the organs with the help of an aseptic razor.
- In some cases, the cells are removed by the use of chemical disintegrators or proteolytic enzymes.
- The cell suspension obtained is washed with buffering liquid in order to remove the proteolytic enzymes.
- The cell suspension is poured onto a flat surface which can be a culture vessel or a sterile Petri plate.
- The cells that can adhere to the base of the vessel are overlaid with an appropriate culture medium and incubated at room temperature.

3. Cell thawing

- In the case of subsequent subcultures, the preserved cell culture might have to be used.
- The water bath is heated to a temperature of 37°C, and the growth media where the cells are to be plated is warmed.
- The warm medium is added to the culture vessel. The vial with the frozen cells is then placed in the water bath until thawed.
- After thawing, the via is washed with 70% alcohol on the outside. The cell suspension is pipetted into the cell culture vessel and swirled gently to mix everything.
- The medium is then incubated overnight under the usual growth conditions. The growth medium is replaced the next day.

4. Trypsinizing Cells

- Trypsinization is the method of separating adherent cells from the surface of the culture vessel with the help of proteolytic enzymes. It is done when the cells are to be used for passaging, counting, or other purposes.
- The medium is removed, and the cells are recovered. The cells are then washed with phosphate buffer.
- Warm trypsin-EDTA is added to the vessel so as to cover the monolayer. The vessel can be rocked to ensure that the monolayer is coated.
- The vessel is incubated in a CO_2 incubator at 37℃ for 1-3 minutes.

- The vessel is removed from the incubator, and the flask is firmly tapped on the side with the palm of the hand to assist detachment.
- Once the cells are dislodged, they are resuspended in an appropriate growth medium containing some amount of serum.
- The cells are then separated with the help of syringe needles by disrupting the cell clumps and used accordingly.

Applications of Animal cell culture

The following are some of the applications of animal cell culture;

a. Production of vaccines

- Animal cell culture is an important technique used for the development of viral vaccine production.
- The technique has been used for the development of a recombinant vaccine against hepatitis B and poliovirus.
- Immortalized cell lines are used for the large-scale or industrial production of viral vaccines.

b. Recombinant proteins

- Animal cell cultures can also be used for the production of recombinant therapeutic proteins like cytokines, hematopoietic growth factors, growth factors, hormones, blood products, and enzymes.
- Some of the common animal cell lines used for the production of these proteins are baby hamster kidney and CHO cells.

c. Gene Therapy

- The development of animal cell culture is critical for the advances in gene therapy.
- Cells with faulty genes can be replaced by a functional gene in order to remove such defects and diseases.

d. Model systems

- Cells obtained from cell culture can be studied as a model system for studies related to cell biology, host-pathogen interactions, effects of drugs, and effects due to changes in the cell composition.

e. Cancer Research

- Animal cell culture can be used to study the differences in cancer cells and normal cells as cancer cells can also be cultured.
- The differences allow more detailed studies on the potential causes and effects of different carcinogenic substances.

- Normal cells can be culture to form cancer cells by the use of certain chemicals, viruses, and radiation.
- Cancer cells can also be used as test systems for studies related to the efficiencies of drugs and techniques used in cancer treatment.

f. Production of Biopesticides

- Animal cell lines like Sf21 and Sf9 can be used for the production of biopesticides due to their faster growth rate and higher cell density.
- Organisms like baculovirus can be produced through animal cell culture as well.

Cell Lines

The development and various other aspects of primary culture are described above. The term cell line refers to the propagation of culture after the first subculture. In other words, once the primary culture is sub-cultured, it becomes a cell line. A given cell line contains several cell lineages of either similar or distinct phenotypes. It is possible to select a particular cell lineage by cloning or physical cell separation or some other selection method. Such a cell line derived by selection or cloning is referred to as cell strain. Cell strains do not have infinite life, as they die after some divisions.

Types of Cell Lines:

Finite Cell Lines:

The cells in culture divide only a limited number of times, before their growth rate declines and they eventually die. The cell lines with limited culture life spans are referred to as finite cell lines. The cells normally divide 20 to 100 times (i.e. is 20-100 population doublings) before extinction. The actual number of doublings depends on the species, cell lineage differences, culture conditions etc. The human cells generally divide 50-100 times, while murine cells divide 30-50 times before dying.

Continuous Cell Lines:

A few cells in culture may acquire a different morphology and get altered. Such cells are capable of growing faster resulting in an independent culture. The progeny derived from these altered cells has unlimited life (unlike the cell strains from which they originated). They are designated as continuous cell lines. The continuous cell lines are transformed, immortal and tumorigenic. The transformed cells for continuous cell lines may be obtained from normal primary cell cultures (or cells strains) by treating them with chemical carcinogens or by infecting with oncogenic viruses. the different properties of finite cell lines and continuous cell lines are compared.

Properties of Finite and Continuous Cell Lines

The most commonly used terms while dealing with cell lines are explained below.

Split ratio:

The divisor of the dilution ratio of a cell culture at subculture. For instance,

when each subculture divided the culture to half, the split ratio is 1: 2.

Passage number:

It is the number of times that the culture has been sub-cultured.

Generation number:

It refers to the number of doublings that a cell population has undergone. It must be noted that the passage number and generation number are not the same, and they are totally different.

Nomenclature of Cell Lines:

It is a common practice to give codes or designations to cell lines for their identification. For instance, the code NHB 2-1 represents the cell line from normal human brain, followed by cell strain (or cell line number) 2 and clone number 1. The usual practice in a culture laboratory is to maintain a log book or computer database file for each of the cell lines. While naming the cell lines, it is absolutely necessary to ensure that each cell line designation is unique so that there occurs no confusion when reports are given in literature. Further, at the time of publication, the-cell line should be prefixed with a code designating the laboratory from which it was obtained e.g. NCI for National Cancer Institute, Wl for Wistar Institute. Commonly used cell lines:

There are thousands of cell lines developed from different laboratories world over. A selected list of some commonly used cell lines along with their origin,

Commonly Used Cell Lines

Selection of Cell Lines:

Several factors need to be considered while selecting a cell line. Some of them are briefly described:

1. Species:

In general, non-human cell lines have less risk of biohazards, hence preferred. However, species differences need to be taken into account while extrapolating the data to humans.

2. Finite or continuous cell lines:

Cultures with continuous cell lines are preferred as they grow faster, easy to clone and maintain, and produce higher yield. But it is doubtful whether the continuous cell lines express the right and appropriate functions of the cells. Therefore, some workers suggest the use of finite cell lines, although it is difficult.

3. Normal or transformed cells:

The transformed cells are preferred as they are immortalized and grow rapidly.

4. Availability:

The ready availability of cell lines is also important. Sometimes, it may be necessary to develop a particular cell line in a laboratory.

5. Growth characteristics:

The following growth parameters need to be considered:

i. Population doubling time

ii. Ability to grow in suspension

iii. Saturation density (yield per flask)

iv. Cloning efficiency.

6. Stability: The stability of cell line with particular reference to cloning, generation of adequate stock and storage are important.

7. Phenotypic expression: It is important that the cell lines possess cells with the right phenotypic expression.

Maintenance of Cell Cultures:

For the routine and good maintenance of cell lines in culture (primary culture or subculture) the examination of cell morphology and the periodic change of medium are very important.

Cell Morphology:

The cells in the culture must be examined regularly to check the health status of the cells, the absence of contamination, and any other serious complications (toxins in medium, inadequate nutrients etc.). Replacement of Medium:

Periodic change of the medium is required for the maintenance of cell lines in culture, whether the cells are proliferating or non-proliferating. For the proliferating cells, the medium need to be changed more frequently when compared to non-proliferating cells. The time interval between medium changes depends on the rate of cell growth and metabolism. For instance, for rapidly growing transformed cells (e.g. HeLa), the medium needs to be changed twice a week, while for slowly growing non-transformed cells (e.g. IMR-90) the medium may be changed once a week. Further, for rapidly proliferating cells, the sub-culturing has to be done more frequently than for the slowly growing cells.

The following factors need to be considered for the replacement of the medium:

1. Cell concentration:

The cultures with high cell concentration utilize the nutrients in the medium faster than those with low concentration; hence the medium is required to be changed more frequently for the former.

2. A decrease in pH:

A fall in the pH of the medium is an indication for change of medium. Most of the cells can grow optimally at pH 7.0, and they almost stop growing when the pH falls to 6.5. A further drop in pH (between 6.5 and 6.0), the cells may lose their viability. The rate of fall in pH is generally estimated for each cell line with a chosen

medium. If the fall is less than 0.1 pH units per day, there is no harm even if the medium is not immediately changed. But when the fall is 0.4 pH units per day, medium should be changed immediately.

3. Cell type:

Embryonic cells, transformed cells and continuous cell lines grow rapidly and require more frequent sub-culturing and change of medium. This is in contrast to normal cells, which grow slowly.

4. Morphological changes:

Frequent examination of cell morphology is very important in culture techniques. Any deterioration in cell morphology may lead to an irreversible damage to cells. Change of the medium has to be done to completely avoid the risk of cell damage.

Mammalian Cell Culture

Mammalian cell culture is the technique of growing animal cells outside of their original tissue but in a growth medium instead.

Cells are typically grown in a cell culture vessel such as a shake flask placed in an incubator that provides a controlled environment for optimal growth. To maintain cell integrity, the medium contains **nutrients, a carbon source such as glucose, vitamins, amino acids, salts, oxygen and CO2, and growth factors.** Some media contain fetal bovine serum (fbs), but this comes with the downside of limited reproducibility and potential contaminations.

On the other hand, it is also possible to grow cells in a serum-free, chemically defined (CD) medium. This approach can be chosen for CHO cells as well, bringing advantages regarding regulatory compliance and downstream purification.

Application of mammalian cell culture

Mammalian cell culture is applied in various fields of life sciences, for instance:

- Manufacturing of biological products such as antibodies, synthetic hormones, enzymes
- Viral vaccine production to protect people from infectious diseases
- Biochemistry in particular biomedical research, physiology and medicine to investigate how cells work and disease causation
- Gene therapy to monitor and alter cellular mechanisms
- Drug screening for efficacy and toxicity

Mammalian Cell Line

A mammalian cell line is a **population of mammalian cells** that can be grown due to their ongoing cell division. Generally, a normal cell has a limited lifespan and does not divide indefinitely. To generate a cell line, cells must undergo immortalization.

Oftentimes, immortalization processes involve mutations of genes that deregulate the cell cycle or enhance proliferation processes. Natural sources of immortal cell lines are cancerous tissues, from which the **first immortal cells originated**.

A more specialized approach is the **fusion of B cells with myeloma cells** to generate immortalized antibody producing hybridoma cells.

In contrast, stem cells have the natural ability to divide indefinitely, e. g. **embryonic stem cells from blastocysts**.

A differentiation must be made with the **culture of primary cells**, where normal, mortal cells are removed from a tissue (e. g. via biopsy) and then grown in culture media.

These cells undergo a few cell divisions and then begin to die. This limits their applications but they are **more representative of live tissue** compared to immortalized cells. Read more on "How recombinant antibodies are made".

The process of mammalian cell culture

Adherent cells, which require a surface for attachment, are commonly used in mammalian cell culture. The process begins with seeding cells onto culture flasks or dishes containing a suitable growth medium. The medium provides essential supplements like vitamins, amino acids, salts, and growth factors necessary for cell growth and proliferation.

To ensure successful cell passage or cloning, the cells need to reach an optimal density. When cells reach confluence, they form a monolayer, indicating that the culture vessel is densely populated with cells. At this stage, trypsin is commonly used to detach the cells from the substrate for sub culturing or transfer to a new culture vessel.

Maintaining appropriate cell density is crucial for optimal growth and function. Overcrowding can lead to nutrient depletion and compromised cell health, while low cell density may result in decreased viability and impaired cellular activity. Regular monitoring and subculturing are necessary to maintain the desired cell density.

Throughout the culture process, maintaining aseptic conditions is essential to prevent contamination. This includes working in a sterile laminar flow hood, using sterile techniques, and employing antibiotics or antifungal agents when necessary.

Apoptosis

"The term apoptosis can be defined as a natural biological process of programmed cell death in which the cells destroy themselves for maintaining the smooth functioning of the body."
There are two forms of cell death

1. Programmed death of cells called Apoptosis.
2. An uncontrolled death of cells called Necrosis.

Both apoptosis and necrosis occur under different circumstances and involve different steps.

The term apoptosis is derived from the Greek word meaning dropping or falling off. It was first introduced by Kerr, Wyllie, and Currie.

Apoptosis is a biological process which occurs in all multicellular organisms including plants and animals. It removes the cells from the organisms that should no longer be a part of the organism. This process plays a major role in the development of humans and in developing and maintaining a healthy immune system.

On an average, 50 – 80 billion cells die every day in a human adult due to apoptosis. During this biological process, infected cells, pre-cancerous cells and other cancer cells are eliminated successfully and maintain the balance of cells in the human body. Therefore, it is an essential process that is responsible for the normal development of cells, cell cycle maturation and maintaining the regular functions and activities of cells.

Apoptosis occurs in all the vertebrates that have fingers and toes like digits. A slight mistake during apoptosis results in fused toes or fingers. The loss of the tail of a tadpole when it develops into a frog is yet another example of apoptosis.

Apoptosis Pathways

The process of apoptosis undergoes two pathways:

- Extrinsic Pathway
- Intrinsic Pathway

Extrinsic Pathway

This pathway triggers apoptosis in response to external stimuli, like, ligand binding at death receptors on the cell surface. These receptors are members of the Tumor Necrosis Factor gene family. The receptor binding initiates caspase activation.

Intrinsic Pathway

This pathway triggers apoptosis in response to internal stimuli such as biochemical stress, DNA damage and lack of growth factors. This pathway is modulated by two groups of molecules- Bax, and Bcl-2. These groups of molecules determine whether a cell will survive or undergo apoptosis in response to the stimuli.

Significance of Apoptosis

Apoptosis is significant for the following reasons:

1. It helps to maintain homeostasis in the multicellular organisms.
2. Proper size of the body is maintained by apoptosis.
3. Apoptosis maintains the constancy of cell number in an organism.
4. The unwanted cells are eliminated from the body by apoptosis.
5. The dangerous T-lymphocytes are eliminated by apoptosis.
6. Programmed cell death is crucial for cell development.

Role Of Apoptosis

Apoptosis plays an important role in the body of an organism. Following are a few such roles performed by the process:

1. The separation of the fingers during the development of the foetus is due to apoptosis.

2. It results in the closure of the neural tube in the dorsal part.
3. Programmed cell death results in the removal of vestigial remnants such as pronephros.
4. During the determination of sex of the foetus, the Wolffian ducts are removed by cell death.

UNIT -3

Stem cells

"Stem cells are special human cells that can develop into many different types of cells, from muscle cells to brain cells."

Stem cells also have the ability to repair damaged cells. These cells have strong healing power. They can evolve into any type of cell.

Research on stem cells is going on, and it is believed that stem cell therapies can cure ailments like paralysis and Alzheimer's as well. Let us have a detailed look at stem cells, their types and their functions.

Types of cells

Stem cells are of the following different types:

- Embryonic Stem Cells
- Adult Stem Cells
- Induced Pluripotent Stem Cells
- Mesenchymal stem cells

Embryonic Stem Cells

The fertilized egg begins to divide immediately. All the cells in the young embryo are totipotent cells. These cells form a hollow structure within a few days. Cells in one region group together to form the inner cell mass. This contains pluripotent cells that make up the developing foetus.

The embryonic stem cells can be further classified as:

- **Totipotent Stem Cells:** These can differentiate into all possible types of stem cells.
- **Pluripotent Stem Cells:** These are the cells from an early embryo and can differentiate into any cell type.
- **Multipotent Stem Cells:** These differentiate into a closely related cell type. E.g., the hematopoietic stem cells differentiate into red blood cells and white blood cells.

- **Oligopotent Stem Cells:** Adult lymphoid or myeloid cells are oligopotent. They can differentiate into a few different types of cells.
- **Unipotent Stem Cells:** They can produce cells only of their own type. Since they have the ability to renew themselves, they are known as unipotent stem cells. E.g., Muscle stem cells.

Adult Stem Cells

These stem cells are obtained from developed organs and tissues. They can repair and replace the damaged tissues in the region where they are located. For eg., hematopoietic stem cells are found in the bone marrow. These stem cells are used in bone marrow transplants to treat specific types of cancers.

Induced Pluripotent Stem Cells

These cells have been tested and arranged by converting tissue-specific cells into embryonic cells in the lab. These cells are accepted as an important tool to learn about the normal development, onset and progression of the disease and are also helpful in testing various drugs. These stem cells share the same characteristics as embryonic cells do. They also have the potential to give rise to all the different types of cells in the human body.

Mesenchymal Stem Cells

These cells are mainly formed from the connective tissues surrounding other tissues and organs, known as the stroma. These mesenchymal stem cells are accurately called stromal cells. The first mesenchymal stem cells were found in the bone marrow that is capable of developing bones, fat cells, and cartilage.

There are different mesenchymal stem cells that are used to treat various diseases as they have been developed from different tissues of the human body. The characteristics of mesenchymal stem cells depend on the organ from where they originate.

Applications of Stem Cells

Following are the important applications of stem cells:

Tissue Regeneration

This is the most important application of stem cells. The stem cells can be used to grow a specific type

of tissue or organ. This can be helpful in kidney and liver transplants. The doctors have already used the stem cells from beneath the epidermis to develop skin tissue that can repair severe burns or other injuries by tissue grafting.

Treatment of Cardiovascular Disease

A team of researchers have developed blood vessels in mice using human stem cells. Within two weeks of implantation, the blood vessels formed their network and were as efficient as the natural vessels.

Treatment of Brain Diseases

Stem cells can also treat diseases such as Parkinson's disease and Alzheimer's. These can help to replenish the damaged brain cells. Researchers have tried to differentiate embryonic stem cells into these types of cells and make it possible to treat diseases.

Blood Disease Treatment

The adult hematopoietic stem cells are used to treat cancers, sickle cell anaemia, and other immunodeficiency diseases. These stem cells can be used to produce red blood cells and white blood cells in the body.

Sources of Stem Cells

Stem Cells originate from different parts of the body. Adult stem cells can be found in specific tissues in the human body. Matured cells are specialized to conduct various functions. Generally, these cells can develop the kind of cells found in tissues where they reside.

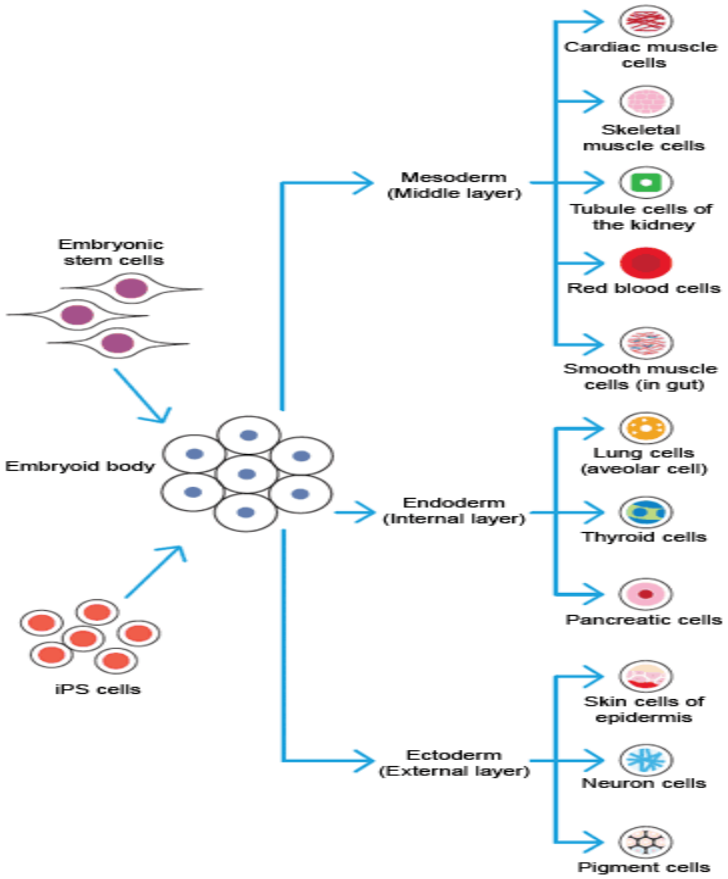
Embryonic Stem Cells are derived from 5-day-old blastocysts that develop into embryos and are pluripotent in nature. These cells can develop any type of cell and tissue in the body. These cells have the potential to regenerate all the cells and tissues that have been lost because of any kind of injury or disease.

Differentiation of Stem Cells

The hallmark of stem cells is their ability to self-renew and to differentiate into different cell types. The induction of stem cell differentiation into a given cell type often requires a specific combination of media and factors. This section provides a brief overview of methods for differentiating human embryonic stem cells (hESCs) into different cell types.

Methods for Stem Cell Differentiation

As with culturing stem cells, methods of differentiation depend on the type of stem cell, the species, target lineages, and somatic cell types. When stem cells are being induced to differentiate, it is essential that the progress be tracked and that the phenotype of the cells be confirmed. The lineages and identities of differentiated cell types can be analyzed using PCR techniques such as real-time PCR or digital PCR, cell sorting/flow cytometry, immunocytochemistry, western blotting, and biomarker analysis.



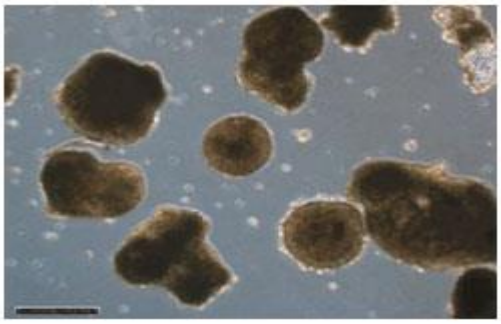
ESCs and induced pluripotent stem cells (iPSC) can form embryoid bodies, which can differentiate into cells of the ectoderm, mesoderm, and endoderm.

The Embryoid Body

-

- One of the oldest methods for stem cell differentiation is the generation of embryoid bodies (EBs). Generally, when stem cells are cultured without an adherent surface, feeder cells, or a complex matrix, the cells aggregate. These aggregated cells spontaneously differentiate. An EB contains all three germ layers.
- EBs are still often used as the initial stage of differentiation for embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). All downstream differentiated cells are derived from this initial structure. There are several methods for forming EBs, including suspension culture, hanging-drop culture, and culture in semisolid media. All protocols start with detaching a high-density cell culture from the dish, either enzymatically or with versine, depending on the properties of the cells.
- **Suspension culture** is the most common method for EB formation but also the hardest to control, for both EB size and shape; EBs can become large and irregularly shaped in suspension. Suspension culture is scalable to bioreactors, although the exact methods and factors such as stir rate that affect the physical dimensions of EBs must be determined empirically.

- **Hanging-drop** culture gives smaller and much more uniform EBs. The size of the EBs can be controlled by controlling the number of cells in each drop. Making the drops (usually ~20 μl) was formally labor intensive, and hence the numbers of EBs generated was low. Now, higher-throughput hanging-drop methods using arrays have been developed and used for anticancer-drug sensitivity testing (Hsaio et al. 2012).
- **Semisolid media**, usually methylcellulose, can be used to suspend the EBs. This method is efficient for making EBs, but is not as scalable as suspension culture.
- ESCs form EBs in about four days, and are often allowed to grow for weeks. All three germ layers are present, but the composition of the media influences the ratios of cell types and lineages.



Depending on the desired end point, the presence of all three germ layers in EBs can be a disadvantage. When a specific lineage or cell type is required, cultures must be depleted of the unwanted cell types. Therefore, many protocols have been developed for directly differentiating ESCs and iPSCs without using EBs. The methods are specific for the species, lineage, and cell type.

Cells of the Ectoderm

- The central nervous system, hair, and the epidermis are all derived from the ectoderm. There are several protocols for producing neural progenitor cells from undifferentiated cultures. One of these protocols is described below (Zhang et al. 2001). This protocol has been the basis for the generation of a number of different neuronal cell types.
- To induce EBs to form neurons, the culture medium is replaced with neural basal media containing bFGF (basic fibroblast growth factor) heparin, and N2 supplement. N2 supplement consists of transferrin, insulin, progesterone, putrescine, and selenite. Two days later, attachment of the differentiating EBs is induced by plating them onto dishes coated with laminin or polyornithine. After an additional 10–11 days in culture, the EBs differentiate into primitive neuroepithelial cells. The identity of the cells can be confirmed by

staining for PAX6 (paired box protein 6, a transcription factor), SOX2 (sex-determining region Y-box 2, another transcription factor), and N-cadherin (a calcium-dependent cell adhesion molecule specific to neural tissue).

- At this point, it is possible to differentiate the neuroepithelial cells into specific cell types of the central nervous system including motor neurons (Li et al. 2005), dopaminergic neurons (Yan et al. 2005), and oligodendrocytes (Nistor et al. 2005).

Cells of the Mesoderm

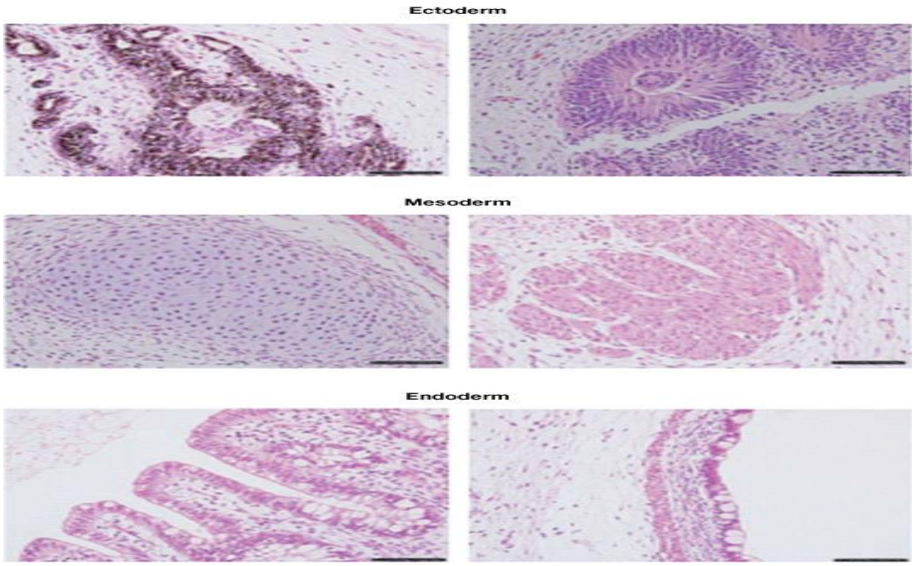
- Cells of the mesoderm form most of the body's internal supporting structures, including blood, muscle, bone, cartilage, and heart. Because new mesodermal cells have potential for use in the treatment of common ailments such as osteoarthritis, osteoporosis, and cardiovascular disease, there has been a major focus on protocols for stem cell differentiation into mesodermal cells.
- Cardiomyocytes develop spontaneously from 10-day-old EBs plated onto gelatin-coated plates (Kehat et al. 2001). Fortunately, although they are a small percentage of the cells, cardiomyocytes are easy to identify due to their hallmark rhythmic contractions. Cardiomyocytes can be separated from the rest of the differentiating culture by cell sorting using antibodies against cardiac markers (Xu et al. 2002).
- An alternative method for deriving cardiomyocytes is to transfect a stem cell culture with a viral vector containing a drug-resistance gene driven by the alpha-myosin heavy chain promoter. Subsequent selection for drug resistance enables the selection of cells that differentiate into cardiomyocytes (Zhao and Lever 2007).
- The differentiation of stem cells into cells within the hematopoietic lineage has long been of important clinical interest for cancers of the blood, such as leukemia. Early work led to the development of techniques

- for differentiating human embryonic stem cells (hESCs) cells into most of the cells of the hematopoietic system (Keller et al. 1993, Kaufman et al. 2001). More recently , the potential for creating replacement cells for blood transfusion, blood cell disease, vascular disease (with endothelial cells), and immunodeficiency disorders has increased interest in the development of techniques for differentiating both iPSCs and hESC into cells of the hematopoietic and vascular systems, and clinical trials have been undertaken for stem cell-derived therapies for leukemia, lymphoma, and sickle cell disease (Nature Biotechnology News 2014).

Cells of the Endoderm

- The endoderm forms many of the internal organs, including the pancreas and the liver. High rates of diabetes and liver disease have made the production of insulin-secreting cells and hepatocytes key goals in the field of stem cell research.

- Type 1 diabetes is caused by the destruction of the insulin-secreting beta cells of the Islet of Langerhans in the pancreas. Current treatment options include pancreatic transplants or the infusion of donor beta cells. However, donors are in short supply, and beta cell transplantation is usually not a permanent cure due to immune response in the recipient, leading to destruction of the donated beta cells.
- It is now possible to make human embryonic stem cells into all pancreatic cell lineages. However, the beta-like cells produced during the complex differentiation process are not efficient insulin producers and are not as completely responsive to cell signaling as native beta cells. A breakthrough in this line of research was recently reported in which large numbers of functional human pancreatic β cells were generated in vitro, providing an unprecedented cell source for drug discovery and cell transplantation therapy in diabetes. Progress toward developing liver cells (hepatocytes) for transplantation has been slow. Stem cells have been differentiated into hepatocyte-like cells using several methods but are generally not suitable for transplantation.



Conclusion

The ability of stem cells to differentiate into nearly any cell in the body gives them the potential to form the basis of therapies for many conditions. As research moves forward, standardized techniques for stem cell culture and differentiation will be developed. These new techniques will lay the foundation for research into cells and tissues and future stem cell therapies.

Knockout mouse

A **knockout mouse** is a genetically engineered mouse that has had one or more of its genes made inoperable through a gene knockout. Knockout is a route to learning about a gene that has been sequenced but has an unknown or incompletely known function. By inactivating the gene and studying the mouse for any resulting differences, researchers can infer the probable function of that gene. Mice are the laboratory animal species most closely related to humans in which the knockout technique can be easily performed, so they are a favorite subject for knockout experiments, especially with regard to genetic questions that relate to human physiology. Gene knockout in rats is much harder[*citation needed*] and has only been possible since 2003.

The first knockout mice were produced by Mario R. Capecchi, Martin Evans and Oliver Smithies in 1987–1989, for which they were awarded the Nobel Prize for Medicine in 2007. Aspects of this technology were licensed to Lexicon Pharmaceuticals. The various methods for generating Knockout mice are extensively patented in the United States. The resulting knockout mice can also be patented in many countries.

Uses

Knocking out the activity of a gene provides information about what that gene normally does. Humans share many genes with mice. Consequently, observing the characteristics of knockout mice gives researchers information that can be used to better understand how a similar gene may cause or contribute to disease in humans.

Examples of research in which knockout mice have been useful include studying and modeling different kinds of cancer, obesity, heart disease, diabetes, arthritis, substance abuse, anxiety, aging and Parkinson's disease. Knockout mice also offer a biological and scientific context in which drugs and other therapies can be developed and tested.

Many of these mouse models are named after the gene that has been inactivated. For example, the p53 knockout mouse is named after the p53 gene which codes for a protein that normally suppresses the growth of tumors by arresting cell division. Humans born with mutations that inactivate the p53 gene suffer from Li-Fraumeni syndrome, a condition that dramatically increases the risk of developing bone cancers, breast cancer and blood cancers at an early age. Other mouse models are named, often with creative flair, according to their physical characteristics or behaviors. For example, "Methuselah" is a knockout mouse model noted for longevity, while "Frantic" is a model useful for studying anxiety disorders.

Procedure

There are several variations to the procedure of producing knockout mice; the following is a typical example.

1. The gene to be knocked out is isolated from a mouse gene library. Then a new DNA sequence is engineered which is very similar to the original gene and its immediate neighbor sequence, except that it is changed sufficiently to make it inoperable. Usually, the new sequence is also given a marker gene, a gene that normal mice don't have and that confers resistance to a certain toxic agent or that produces an observable change (e.g. colour or flurorescence). The chances of a successful recombination event are relatively low, so the majority of altered cells will have the gene changed for only one of the two relevant chromosomes - they are said to be heterozygous.
2. From a mouse blastocyst (a very young embryo consisting of a ball of undifferentiated cells with surrounding extraembryonic cells), stem cells are isolated; these can be grown *in vitro*. For this example, we will take a stem cell from a white mouse.
3. The stem cells from step 2 are combined with the new sequence from step 1. This is done via electroporation (using electricity to transfer the DNA across the cell membrane). Some of the electroporated stem cells will incorporate the new sequence into their chromosomes in place of the old gene; this is called homologous recombination. The reason for this process is that the new and the old sequence are very similar. Using the marker gene from step 1, those stem cells that actually did incorporate the new sequence can be quickly isolated from those that did not.
4. The stem cells from step 3 are inserted into a mouse blastocyst. For this example, we use blastocysts from a grey mouse. These blastocysts are then implanted into the uterus of female mice, to complete the pregnancy. The blastocysts contain two types of stem cells: the original ones (grey mouse), and the newly engineered ones (white mouse). The newborn mice will therefore be chimeras: parts of their bodies result from the original stem cells, other parts result from the engineered stem cells. Their furs will show patches of white and grey.
5. Newborn mice are only useful if the newly engineered sequence was incorporated into the germ cells (egg or sperm cells). These new mice are crossed with others of the white type for offspring that are all white. These mice still contain one functional copy of the gene and must be further inbred to produce mice that carry no functional copy of the original gene (i.e. are homozygous for that allele).

Limitations

While knockout mice technology represents a valuable research tool, some important limitations exist. About 15 percent of gene knockouts are developmentally lethal, which means that the genetically altered embryos cannot grow into adult mice. This problem is often overcome through the use of conditional mutations. The lack of adult mice limits studies to embryonic development and often makes it more difficult

to determine a gene's function in relation to human health. In some instances, the gene may serve a different function in adults than in developing embryos.

Knocking out a gene also may fail to produce an observable change in a mouse or may even produce different characteristics from those observed in humans in which the same gene is inactivated. For example, mutations in the p53 gene are associated with more than half of human cancers and often lead to tumors in a particular set of tissues. However, when the p53 gene is knocked out in mice, the animals develop tumors in a different array of tissues.

UNIT – 4

Hematopoiesis

All blood cells are produced by a mechanism known as hematopoiesis, arising from a single cell type known as a hematopoietic stem cell (HSC). Stem cells are cells that differentiate into other cell types; they are self-renewing-maintaining their population level by cell division.

Hematopoietic Stem Cell Lineages and Growth Factors

- In humans, hematopoietic formation and development of red and white blood cells being in the embryonic yolk sac in the first week of development. The yolk-sac stem cells differentiate into primitive erythroid cells that contain embryonic hemoglobin.
- During the third month of the gestation period, the hematopoietic stem cells migrate from the yolk sac to the fetal liver and the spleen, which are the major organs from hematopoiesis from the 3rd to the 7th month of the gestation. Then the hematopoietic process of the stem cells then starts in the bone marrow until birth when there is little or no hematopoiesis in the liver and spleen.
- Therefore, every mature and specialized blood cell is derived from the same type of stem cell, a type of cell lineage known as multipotent or pluripotent hematopoietic stem cells. These are stem cells that are able to differentiate and generate various cell types including erythrocytes, granulocytes, monocytes, mast cells, lymphocytes, and megakaryocytes. However, their numbers are few (1 HSC in every 5104 cells in the bone marrow).
- Hematopoietic stem cells remain at a stable level throughout adult life and research has shown that their proliferation capacity is enormous.
- In the early phase of hematopoiesis, the multipotent stem cells differentiate along with one of the two pathways, which gives rise to either a common lymphoid progenitor cell or a common myeloid progenitor cell, which is dictated by its microenvironment.
- During the development of the lymphoid and myeloid lineages, stem cells differentiate into progenitor cells, which have lost the capacity for self-renewal and are committed to a particular cell lineage.
- Common lymphoid progenitor cells give rise to B-cells, T-cells, NK (natural killer) cells, and some dendritic cells.
- Myeloid stem cells generate progenitors of red blood cells (erythrocytes), many of the various white blood cells (neutrophils, eosinophils, basophils, monocytes, mast cells, dendritic cells), and platelets.

- The progenitor productions are highly dependent on the acquisition of response to growth factors and cytokines, which help in the proliferation and differentiation of the progenitor cells into the corresponding cell types, which can either be a mature erythrocyte, a specific type of leukocyte, or a platelet-generating cell (megakaryocytes).

- The Red blood cells and the white blood cells pass into the bone marrow and through the circulation. Within the bone marrow, the hematopoietic cells grow and mature on a mesh of stromal cells which are non-hematopoietic cells supporting the growth and differentiation of hematopoietic cells.
- The stromal cells include fat cells, endothelial cells, fibroblasts, and macrophages, and they influence the process of hematopoietic differentiation by providing a hematopoietic-inducing microenvironment (HIM). The HIM consists of a cellular matrix and factors that promote growth and differentiation.
- The hematopoietic growth factors normally include soluble agents that arrive at their target cells by diffusion and other membrane-bound molecules on the surface of stromal cells that require cell-to-cell contact between the responding cells and the stromal cells.
- When the body has an infection or during infection, hematopoiesis is stimulated by the production of hematopoietic growth factors by activated macrophages and T-cells.
- Research on the genetic level of hematopoiesis identifies some of the growth factors involved to be transcriptional factors, that affect the hematopoietic lineages and other factors that influence a single lineage which leads to the development of lymphocytes.
- One transcription factor that affects multiple lineages is, GATA-2, a transcriptional factor that recognizes the tetranucleotide sequence GATA, a nucleotide motif in target genes. A functional GATA-2 gene, which specifies this transcription factor, is essential for the development of the lymphoid, erythroid, and myeloid lineages.

Regulation of Hematopoiesis and Programmed Cell death

- The hematopoietic process is a continuous process to maintain a steady and constant production of mature blood cells to balance production to that of loss by cell-aging. RBCs (erythrocytes) have an average life span of 120 days before it is phagocytosed and digested by spleen macrophages.
- The categories of white blood cells have a life span of few days like neutrophils, to over 20 years for some T-lymphocytes, and therefore to maintain a steady-state level, a human being must produce at least 3.7×1011 white blood cells per day.
- The regulation of hematopoiesis is done by several complex mechanisms that affect all individual cell types. These regulatory mechanisms ensure steady-state levels of the various blood cells yet they have enough built-in flexibility so that the production of blood cells can rapidly increase tenfold to twenty-fold in response to hemorrhage or infection.
- Steady-state regulation of hematopoiesis is accomplished in various ways, which include:
- Control of the levels and types of cytokines produced by bone marrow stromal cells

- The production of cytokines with hematopoietic activity by other cell types, such as activated T cells and macrophages
- The regulation of the expression of receptors for hematopoietically active cytokines in stem cells and progenitor cells
- The removal of some cells by the controlled induction of cell death
- A failure in one or a combination of these regulatory mechanisms can cause extreme abnormalities in expressing the hematopoietic cytokines or their receptors. This can lead to unregulated cell proliferation and it can contribute to the development of some types of leukemias.
- Therefore there should be a balance between the number of hematopoietic lineages production (differentiation and proliferation) and the number of cells that are removed by cell death.
- Lastly, hematopoietic cell lineages undergo programmed cell death known as apoptosis. Each of the immune cells has a specific life span after which they naturally die by what is referred to as programmed cell death. For example, the neutrophils are about 5×1010 in circulation, and they have a life span of a few days before programmed cell death is initiated. This death along with constant neutrophil production maintains a stable number of the cells.
- However, if programmed cell death occurs, a leukemic state may develop.

Cord Blood Stem Cell Transplantation

Umbilical cord blood, like bone marrow and peripheral blood, is a rich source of stem cells for transplantation. There may be advantages for certain patients to have cord blood stem cell transplants instead of transplants with marrow or peripheral blood stem cells (PBSCs).

- Stem cell transplants (peripheral blood, marrow or cord blood) may use the patient's own stem cells (called "autologous transplants") or use donor stem cells. Donor cells may come from either a related or unrelated matched donor (called an "allogeneic transplant"). Most transplant physicians would not want to use a baby's own cord blood ("autologous transplant") to treat his or her leukemia. This is because donor stem cells might better fight the leukemia than the child's own stem cells.
- Cord blood for transplantation is collected from the umbilical cord and placenta after a baby is delivered. Donated cord blood that meets requirements is frozen and stored at a cord blood bank for future use.

- The American Academy of Pediatrics's (AAP) policy statement (Pediatrics; 2007;119:165-170.) addresses public and private banking options available to parents. Among several recommendations, the report encourages parents to donate to public cord blood banks and discourages parents from

using private cord blood banks for personal or family cord blood storage unless they have an older child with a condition that could benefit from transplantation.

- The Stem Cell Therapeutic and Research Act of 2005 put several programs in place, including creation of the National Cord Blood Inventory (NCBI) for patients in need of transplantation.
- Research studies of cord blood transplant outcomes, including transplants with two or more cord blood units, show promising results
- Stem Cell Transplantation The transplantation of blood-forming stem cells is an accepted treatment to restore the body's ability to make blood and immune cells. Blood-forming or "hematopoietic" stem cells (HSCs) can develop into any of the three types of blood cells: red cells, white cells or platelets.
- Cancer treatment with very high-dose chemotherapy or radiation therapy may result in severe injury to blood-forming cells in marrow, the spongy material inside the center of bones. Certain patients with leukemia, lymphoma, myeloma, myelodysplastic syndromes (MDS) or other blood cancers may benefit from high-dose chemotherapy or radiation therapy followed by stem cell transplantation. Patients with some other inherited or acquired marrow or immune system disorders may also benefit from stem cell transplantation.

Sources of Stem Cells for Transplantation

- The cells used in transplants can come from three sources: marrow, peripheral blood and the blood in the umbilical cord after a baby's birth.
- To obtain cells from marrow, the physician removes marrow from a donor's hip bone in a surgical procedure using anesthesia, sterile needles and syringes. The donor's body replaces the donated bone marrow in four to six weeks.
- Currently, peripheral blood is the most common source of stem cells for transplant. Peripheral blood stem cells (PBSCs) are blood-forming stem cells released from the marrow into the bloodstream. Peripheral blood is also called "circulating blood." Normally, the marrow releases only a small number of these stem cells into the blood. To obtain enough stem cells from the peripheral blood for a transplant, a donor is given medication that encourages more blood-forming stem cells to move from the marrow to the blood. The cells are collected from the blood using a process called "apheresis." For apheresis, a needle is placed in the donor's vein, usually in the arm. The donor's blood passes through a machine that removes the stem cells and then returns the rest of the blood to the donor. The donor's body replaces the cells in two to three weeks.
- Cord blood stem cells are collected from the umbilical cord and placenta after a baby is born. Cord and placental blood contain large numbers of blood-forming stem cells. The donated cord blood is screened, frozen and stored at a cord blood bank for future use if it meets screening requirements.

The stored cord blood collected from the umbilical cord and placenta after a baby is born is called a "cord blood unit."

Cord Blood Stem Cell Transplants

- The first successful cord blood stem cell transplant was performed in 1988 in Paris, France..
- Cord blood transplants can have complications similar to allogeneic stem cell transplants and should be done only at centers experienced in the transplantation of allogeneic sources of stem cells.

Potential Advantages for Patients For certain patients

- there may be advantages to using donor cord blood stem cells instead of donor peripheral blood or donor marrow stem cells. Some potential advantages are
- **Availability**. Cord blood stored in a public cord blood bank has been prescreened, tested and frozen and is ready to use; on the other hand, it can take several months to find and confirm a marrow or peripheral blood donor.
- **Human Leukocyte Antigen (HLA) Matching**. The outcomes of related and unrelated donor stem cell transplants are strongly affected by the degree of HLA matching between the transplant recipient and the donor cord blood. HLA matching plays an important role in successful engraftment, severity of graft-versus-host disease (GVHD) and overall survival. A close match between the patient and the cord blood unit can improve a patient's outcome after transplantation. However, even though a closely matched cord blood unit is preferred, clinical studies suggest that the match may not have to be as close as the match that is necessary for bone marrow or peripheral blood transplants.
- **Graft-Versus-Host Disease**. Studies have found that after a cord blood stem cell transplant, fewer patients got GVHD and, among those patients who did develop GVHD, the complication tended to be less severe than it was in patients who had bone marrow or peripheral blood transplants. GVHD is a serious and sometimes fatal complication of allogeneic stem cell transplantation. With GVHD, the donor's immune cells (the graft) attack the patient's healthy tissue (the host).
- **Diversity**. As a result of extending collection efforts to hospitals where births from diverse ethnic backgrounds are well represented, donated cord blood units have the potential to provide a source of stem cells that reflects racial diversity.
- **Infectious Disease Transmission**. Cord blood stem cell transplants carry less risk of transmission of blood-borne infectious diseases compared with stem cells from the peripheral blood or marrow of related or unrelated donors.

Bone Marrow Transplantation

Bone marrow is the soft, spongy tissue found inside bones. It is where most of the body's blood cells develop and are stored.

The blood cells that make other blood cells are called *stem cells*. The most primitive of the stem cells is called the *pluripotent stem cell*. This is different than other blood cells with regard to the following properties:

- **Renewal.** It is able to reproduce another cell identical to itself.
- **Differentiation.** It is able to generate one or more subsets of more mature cells.

It is the stem cells that are needed in bone marrow transplant.

A bone marrow transplant can be used to:

- Replace diseased, nonfunctioning bone marrow with healthy functioning bone marrow (for conditions such as leukemia, aplastic anemia, and sickle cell anemia).
- Regenerate a new immune system that will fight existing or residual leukemia or other cancers not killed by the chemotherapy or radiation used in the transplant.
- Replace the bone marrow and restore its normal function after high doses of chemotherapy and/or radiation are given to treat a malignancy. This process is often called *rescue.*
- Replace bone marrow with genetically healthy functioning bone marrow to prevent more damage from a genetic disease process (such as Hurler's syndrome and adrenoleukodystrophy).

The risks and benefits must be weighed in a thorough discussion with your healthcare provider and specialists in bone marrow transplants before the procedure.

The following diseases are the ones that most commonly benefit from bone marrow transplant:

- Leukemias
- Severe aplastic anemia
- Lymphomas
- Multiple myeloma
- Immune deficiency disorders
- Some solid-tumor cancers (in rare circumstances)

However, patients experience diseases differently, and bone marrow transplant may not be right for everyone who suffers from these diseases.

Types of bone marrow transplants

There are different types of bone marrow transplants depending on who the donor is. The different types of BMT include the following:

- **Autologous bone marrow transplant.** The donor is the patient himself or herself. Stem cells are taken from the patient either by bone marrow harvest or apheresis (a process of collecting peripheral blood stem cells), frozen, and then given back to the patient after intensive treatment. Often the term *rescue* is used instead of *transplant*.
- **Allogeneic bone marrow transplant.** The donor shares the same genetic type as the patient. Stem cells are taken either by bone marrow harvest or apheresis from a genetically matched donor, usually a brother or sister. Other donors for allogeneic bone marrow transplants may include the following:
- **A parent.** A haploid-identical match is when the donor is a parent and the genetic match is at least half identical to the recipient. These transplants are rare.
- **Unrelated bone marrow transplants (UBMT or MUD for matched unrelated donor).** The genetically matched marrow or stem cells are from an unrelated donor. Unrelated donors are found through national bone marrow registries.
- **Umbilical cord blood transplant.** Stem cells are taken from an umbilical cord immediately after delivery of an infant. These stem cells reproduce into mature, functioning blood cells quicker and more effectively than do stem cells taken from the bone marrow of another child or adult. The stem cells are tested, typed, counted, and frozen until they are needed for a transplant.

The bone marrow transplant procedure

The preparations for a bone marrow transplant vary depending on the type of transplant, the disease needing transplant, and your tolerance for certain medicines. Consider the following:

- Most often, high doses of chemotherapy and/or radiation are included in the preparations. This intense therapy is required to effectively treat the malignancy and make room in the bone marrow for the new cells to grow. This therapy is often called ablative, or myeloablative, because of the effect on the bone marrow. The bone marrow produces most of the blood cells in our body. Ablative therapy prevents this process of cell production and the marrow becomes empty. An empty marrow is needed to make room for the new stem cells to grow and establish a new blood cell production system.

- After the chemotherapy and/or radiation is administered, the marrow transplant is given through the central venous catheter into the bloodstream. It is not a surgical procedure to place the marrow into

the bone, but is similar to receiving a blood transfusion. The stem cells find their way into the bone marrow and begin reproducing and growing new, healthy blood cells.

- After the transplant, supportive care is given to prevent and treat infections, side effects of treatments, and complications. This includes frequent blood tests, close monitoring of vital signs, strict measurement of fluid input and output, daily weigh-ins, and providing a protected and clean environment.

The days before transplant are counted as minus days. The day of transplant is considered day zero. Engraftment and recovery following the transplant are counted as plus days. For example, a patient may enter the hospital on day -8 for preparative regimen. The day of transplant is numbered zero. Days +1, +2, etc., will follow. There are specific events, complications, and risks associated with each day before, during, and after transplant. The days are numbered to help the patient and family understand where they are in terms of risks and discharge planning.

During infusion of bone marrow, the patient may experience the following:

- Pain
- Chills
- Fever
- Hives
- Chest pain

After infusion, the patient may:

- Spend several weeks in the hospital
- Be very susceptible to infection
- Experience excessive bleeding
- Need blood transfusions
- Be confined to a clean environment
- Take multiple antibiotics and other medicines
- Be given medicine to prevent graft-versus-host disease—if the transplant was allogeneic. The transplanted new cells (the graft) tend to attack the patient's tissues (the host), even if the donor is a relative.

- Undergo continual laboratory testing

- Experience nausea, vomiting, diarrhea, mouth sores, and extreme weakness
- Experience temporary mental confusion and emotional or psychological distress

After leaving the hospital, the recovery process continues for several months or longer, during which time the patient cannot return to work or many previously enjoyed activities.

Side effects

Complications may vary, depending on the following:

- Type of marrow transplant
- Type of disease requiring transplant
- Preparative regimen
- Age and overall health of the recipient
- Variance of tissue matching between donor and recipient
- Presence of severe complications

The following are complications that may happen with a bone marrow transplant. However, each individual may experience symptoms differently. These complications may also happen alone, or in combination:

- Infections. Infections are likely in the patient with severe bone marrow suppression. Bacterial infections are the most common. Viral and fungal infections can be life-threatening. Any infection can cause an extended hospital stay, prevent or delay engraftment, and/or cause permanent organ damage. Antibiotics, antifungal medicines, and antiviral medicines are often given to try to prevent serious infection in the immunosuppressed patient.
- Low platelets and low red blood cells. Thrombocytopenia (low platelets) and anemia (low red blood cells), as a result of a nonfunctioning bone marrow, can be dangerous and even life-threatening. Low platelets can cause dangerous bleeding in the lungs, gastrointestinal (GI) tract, and brain.
- Pain. Pain related to mouth sores and gastrointestinal (GI) irritation is common. High doses of chemotherapy and radiation can cause severe mucositis (inflammation of the mouth and GI tract).
- Fluid overload. Fluid overload is a complication that can lead to pneumonia, liver damage, and high blood pressure. The main reason for fluid overload is because the kidneys cannot keep up with the large amount of fluid being given in the form of intravenous (IV) medicines, nutrition, and blood products. The kidneys may also be damaged from disease, infection, chemotherapy, radiation, or antibiotics.
- Respiratory distress. Respiratory status is an important function that may be compromised during transplant. Infection, inflammation of the airway, fluid overload, graft-versus-host disease, and bleeding are all potential life-threatening complications that may happen in the lungs and pulmonary system.

- Organ damage. The liver and heart are important organs that may be damaged during the transplantation process. Temporary or permanent damage to the liver and heart may be caused by infection, graft-versus-host disease, high doses of chemotherapy and radiation, or fluid overload.
- Graft failure. Failure of the graft (transplant) taking hold in the marrow is a potential complication. Graft failure may happen as a result of infection, recurrent disease, or if the stem cell count of the donated marrow was insufficient to cause engraftment.

Parkinson's disease

- Parkinson's disease is a brain disorder that causes unintended or uncontrollable movements, such as shaking, stiffness, and difficulty with balance and coordination.
- Symptoms usually begin gradually and worsen over time. As the disease progresses, people may have difficulty walking and talking. They may also have mental and behavioral changes, sleep problems, depression, memory difficulties, and fatigue.
- While virtually anyone could be at risk for developing Parkinson's, some research studies suggest this disease affects more men than women. It's unclear why, but studies are underway to understand factors that may increase a person's risk. One clear risk is age: Although most people with Parkinson's first develop the disease after age 60, about 5% to 10% experience onset before the age of 50. Early-onset forms of Parkinson's are often, but not always, inherited, and some forms have been linked to specific alterations in genes.

Causes Parkinson's disease

The most prominent signs and symptoms of Parkinson's disease occur when nerve cells in the basal ganglia, an area of the brain that controls movement, become impaired and/or die. Normally, these nerve cells, or neurons, produce an important brain chemical known as dopamine. When the neurons die or become impaired, they produce less dopamine, which causes the movement problems associated with the disease. Scientists still do not know what causes the neurons to die.

People with Parkinson's disease also lose the nerve endings that produce nor epinephrine, the main chemical messenger of the sympathetic nervous system, which controls many functions of the body, such as heart rate and blood pressure. The loss of nor epinephrine might help explain some of the non-movement features of Parkinson's, such as fatigue, irregular blood pressure, decreased movement of food through the digestive tract, and sudden drop in blood pressure when a person stands up from a sitting or lying position.

Many brain cells of people with Parkinson's disease contain Lewy bodies, unusual clumps of the protein alpha-syncline. Scientists are trying to better understand the normal and abnormal functions of alpha-synuclein and its relationship to genetic variants that impact Parkinson's and Lewy body dementia.

Some cases of Parkinson's disease appear to be hereditary, and a few cases can be traced to specific genetic variants. While genetics is thought to play a role in Parkinson's, in most cases the disease does not seem to run in families. Many researchers now believe that Parkinson's results from a combination of genetic and environmental factors, such as exposure to toxins.

Symptoms of Parkinson's disease

Parkinson's has four main symptoms:

- Tremor in hands, arms, legs, jaw, or head
- Muscle stiffness, where muscle remains contracted for a long time
- Slowness of movement
- Impaired balance and coordination, sometimes leading to falls

Other symptoms may include:

- Depression and other emotional changes
- Difficulty swallowing, chewing, and speaking
- Urinary problems or constipation
- Skin problems

The symptoms of Parkinson's and the rate of progression differ among individuals. Early symptoms of this disease are subtle and occur gradually. For example, people may feel mild tremors or have difficulty getting out of a chair. They may notice that they speak too softly, or that their handwriting is slow and looks cramped or small. Friends or family members may be the first to notice changes in someone with early Parkinson's. They may see that the person's face lacks expression and animation, or that the person does not move an arm or leg normally.

People with Parkinson's disease often develop a parkinsonian gait that includes a tendency to lean forward; take small, quick steps; and reduce swinging their arms. They also may have trouble initiating or continuing movement.

Symptoms often begin on one side of the body or even in one limb on one side of the body. As the disease progresses, it eventually affects both sides. However, the symptoms may still be more severe on one side than on the other.

Many people with Parkinson's disease note that prior to experiencing stiffness and tremor, they had sleep problems, constipation, loss of smell, and restless legs. While some of these symptoms may also occur with normal aging, talk with your doctor if these symptoms worsen or begin to interfere with daily living.

Diagnosis of Parkinson's disease

There are currently no blood or laboratory tests to diagnose non-genetic cases of Parkinson's. Doctors usually diagnose the disease by taking a person's medical history and performing a neurological examination. If symptoms improve after starting to take medication, it's another indicator that the person has Parkinson's.

A number of disorders can cause symptoms similar to those of Parkinson's disease. People with Parkinson's-like symptoms that result from other causes, such as multiple system atrophy and dementia with Lewy bodies, are sometimes said to have parkinsonism. While these disorders initially may be misdiagnosed as Parkinson's, certain medical tests, as well as response to drug treatment, may help to better evaluate the cause. Many other diseases have similar features but require different treatments, so it is important to get an accurate diagnosis as soon as possible.

Treatments for Parkinson's disease

Although there is no cure for Parkinson's disease, medicines, surgical treatment, and other therapies can often relieve some symptoms.

Medicines for Parkinson's disease

Medicines can help treat the symptoms of Parkinson's by:

- Increasing the level of dopamine in the brain
- Having an effect on other brain chemicals, such as neurotransmitters, which transfer information between brain cells
- Helping control non-movement symptoms

The main therapy for Parkinson's is levodopa. Nerve cells use levodopa to make dopamine to replenish the brain's dwindling supply. Usually, people take levodopa along with another medication called carbidopa. Carbidopa prevents or reduces some of the side effects of levodopa therapy — such as nausea, vomiting, low blood pressure, and restlessness — and reduces the amount of levodopa needed to improve symptoms.

People living with Parkinson's disease should never stop taking levodopa without telling their doctor. Suddenly stopping the drug may have serious side effects, like being unable to move or having difficulty breathing.

The doctor may prescribe other medicines to treat Parkinson's symptoms, including:

- Dopamine agonists to stimulate the production of dopamine in the brain
- Enzyme inhibitors (e.g., MAO-B inhibitors, COMT inhibitors) to increase the amount of dopamine by slowing down the enzymes that break down dopamine in the brain
- Amantadine to help reduce involuntary movements
- Anticholinergic drugs to reduce tremors and muscle rigidity

Deep brain stimulation

For people with Parkinson's disease who do not respond well to medications, the doctor may recommend deep brain stimulation. During a surgical procedure, a doctor implants electrodes into part of the brain and

connects them to a small electrical device implanted in the chest. The device and electrodes painlessly stimulate specific areas in the brain that control movement in a way that may help stop many of the movement-related symptoms of Parkinson's, such as tremor, slowness of movement, and rigidity.

Other therapies

Other therapies that may help manage Parkinson's symptoms include:

- Physical, occupational, and speech therapies, which may help with gait and voice disorders, tremors and rigidity, and decline in mental functions
- A healthy diet to support overall wellness
- Exercises to strengthen muscles and improve balance, flexibility, and coordination
- Massage therapy to reduce tension
- Yoga and tai chi to increase stretching and flexibility

Alzheimer's disease

- Alzheimer's disease is a condition that affects the brain. The symptoms are mild at first and become more severe over time. It is named after Dr. Alois Alzheimer, who first described the condition.
- Common symptoms of Alzheimer's disease include memory loss, language problems, and impulsive or unpredictable behavior.
- One of the main features of the condition is the presence of plaques and tangles in the brain. Another feature is a loss of connection between the nerve cells, or neurons, in the brain.
- These features mean that information cannot pass easily between different areas of the brain or between the brain and the muscles or organs.
- As the symptoms worsen, it becomes harder for people to remember recent events, to reason, and to recognize people they know. Eventually, a person with Alzheimer's disease may need full-time assistance.

Symptoms

Alzheimer's disease is a progressive condition, meaning that the symptoms get worse over time. Memory loss is a key feature, and this tends to be one of the first symptoms to develop.

The symptoms appear gradually, over months or years. If they develop over hours or days, a person may require medical attention, as this could indicate a stroke.

Symptoms of Alzheimer's disease include:

- **Memory loss:** A person may have difficulty taking in new information and remembering information. This can lead to:
    - repeating questions or conversations

  - losing objects
  - forgetting about events or appointments
  - wandering or getting lost

- **Cognitive deficits:** A person may experience difficulty with reasoning, complex tasks, and judgment. This can lead to:
  - a reduced understanding of safety and risks
  - difficulty with money or paying bills
  - difficulty making decisions
  - difficulty completing tasks that have several stages, such as getting dressed
- **Problems with recognition:** A person may become less able to recognize faces or objects or less able to use basic tools. These issues are not due to problems with eyesight.
- **Problems with spatial awareness:** A person may have difficulty with their balance, trip over, or spill things more often, or they may have difficulty orienting clothing to their body when getting dressed.
- **Problems with speaking, reading, or writing:** A person may develop difficulties with thinking of common words, or they may make more speech, spelling, or writing errors.
- **Personality or behavior changes:** A person may experience changes in personality and behavior that include:
  - becoming upset, angry, or worried more often than before
  - a loss of interest in or motivation for activities they usually enjoy
  - a loss of empathy
  - compulsive, obsessive, or socially inappropriate behavior

Stages

Alzheimer's disease can range from mild to severe. The scale ranges from a state of mild impairment, through to moderate impairment, before eventually reaching severe cognitive decline.

Mild Alzheimer's disease

People with mild Alzheimer's disease develop memory problems and cognitive difficulties that may include the following:

- taking longer than usual to perform daily tasks
- difficulty handling money or paying the bills
- wandering and getting lost
- experiencing personality and behavior changes, such as getting upset or angry more easily, hiding things, or pacing

Moderate Alzheimer's disease

In moderate Alzheimer's disease, the parts of the brain responsible for language, senses, reasoning, and consciousness are damaged. This can lead to the following symptoms:

- greater memory loss and confusion
- difficulty recognizing friends or family
- an inability to learn new things
- difficulty performing tasks with several stages, such as getting dressed
- difficulty coping with new situations
- impulsive behavior
- hallucinations, delusions, or paranoia

Severe Alzheimer's disease

In severe Alzheimer's disease, plaques and tangles are present throughout the brain, causing the brain tissue to shrink substantially. This can lead to:

- an inability to communicate
- dependency on others for care
- being unable to leave bed all or most of the time