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SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY

UNIT – I - Medical Biotechnology – SBB3101

Subject Name: Medical Biotechnology Subject Code: SBB3101

SBB3101	MEDICAL BIOTECHNOLOGY	L	T	P	Credits	Total Marks
		4	0	0	4	100

COURSE OBJECTIVES

- To study about the medicinal approach of Biotechnology and recent advancements in diagnosis.

UNIT 1 ANIMAL CELL CULTURE
12 Hrs.

Animal cell culture-media, maintenance and culture of primary, secondary and continuous cell lines- organ culture-applications- cancer cell lines- apoptosis. Tissue Engineering – Skin, Liver, Pancreas. Assisted reproductive technology- Pregnancy diagnosis.

UNIT 2 CHROMOSOMAL ABNORMALITIES
12 Hrs.

Chromosomal disorders – Gene controlled diseases –Identification of disease genes Haemophilia, DMD, Alzheimer's – Molecular basis of human diseases: Pathogenic mutations – Oncogenes – Loss of function – Tumour Suppressor Genes Immunopathology: Hepatitis, Autoimmune Disorders.

UNIT 3 DIAGNOSTIC TECHNIQUES **12 Hrs.**

Prenatal diagnosis – Invasive techniques and Non-invasive techniques – Diagnosis of pathogenic microbes: Classical and modern methods- Diagnosis using protein and enzyme markers, DNA/RNA based diagnosis – Molecular markers – Microarray technology – genomic and cDNA arrays.

UNIT 4 PREVENTION AND TREATMENT **12 Hrs.**

Vaccines-conventional, recombinant, synthetic peptide, anti-idiotype, DNA vaccines Deletion mutant and vaccinia vector vaccine- Antibiotics-mode of action- antibacterial, antifungal, antiviral, antitumor antibiotics- synthetic chemotherapeutic agent development of microbial resistance to antibiotics.

UNIT 5 MODERN MEDICINE **12 Hrs.**

Hybridoma technique for MCAb production and applications- Gene therapy: Exvivo, In vivo, In situ- Cell and tissue engineering- Stem cell therapy- Nanomedicines- Gene products in medicine – Humulin, Erythropoietin, Growth Hormone/Somatostatin, tPA, Interferon.

Max Hours.60

TEXT / REFERENCE BOOKS:

1. Ramasamy, P. "Trends in Biotechnology", University of Madras, Pearl press, 2002. 2. Trevan. "Biotechnology". Tata McGraw-Hill, 2005.
2. Betty Forbes, Danial SAHM Alics Weinfield, Bailey 2007. Scott's diagnostic microbiology, 12th edition Mosby.
3. Jogdand, S. N. Medical Biotechnology, Himalaya Publishing house, Mumbai, 2005. 2. Click, B. R. and Pasternak. Molecular Biotechnology: Principle and applications of recombinant DNA. ASM Press, 2010.

END SEMESTER EXAMINATION QUESTION PAPER PATTERN

Max. Marks: 100 **Exam Duration: 3 Hrs.**
PART A: 10 questions of 2 marks each - No choice **20 Marks**
PART B: 2 questions from each unit of internal choice; each carrying 16 marks **80 Marks**

Unit 1: Animal Cell Culture

1.1 Animal Cell Culture

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

2. The principal purpose of cell, tissue and organ culture is to isolate, at each level of organization, the parts from the whole organism for study in experimentally controlled environments. It is characteristic of intact organisms that a high degree of interrelationship exists and interaction occurs between the component parts.

3. Cultivation in vitro places cells beyond the effects of the organism as a whole and of the products of all cells other than those introduced into the culture. Artificial environments may be designed to imitate the natural physiological one, or varied at will by the deliberate introduction of particular variables and stresses.

4. Virtually all types of cells or aggregates of cells may be studied in culture. Living cells can be examined by cine photomicrography, and by direct, phase-contrast, interference, fluorescence, or ultraviolet microscopy.

5. Fixed cells from culture are suitable for cytological, cytochemical, histological, histochemical, and electron microscopical study. Populations of cells from monolayers or suspension cultures are used for nutritional, biochemical, and immunological work.

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

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

1.1.1 Maintenance and culture of Primary Cell Lines:

1. A primary culture is that stage of the culture after isolation of the cells but before the first subculture. There are four stages to consider:

    1. Acquisition of the sample
    2. Isolation of the tissue
    3. Dissection and/or disaggregation
    4. Culture after seeding into the culture vessel

2. After isolation, a primary cell culture may be obtained either by allowing cells to migrate out from fragments of tissue adhering to a suitable substrate or by disaggregating the tissue mechanically or enzymatically to produce a suspension of cells, some of which will ultimately attach to the substrate. It appears to be essential for most normal untransformed cells, with the exception of hematopoietic cells, to attach to a flat surface in order to survive and proliferate with maximum efficiency.

3. Transformed cells, on the other hand, particularly cells from transplantable animal tumors, are often able to proliferate in suspension. The enzymes used most frequently for tissue disaggregation are crude preparations of trypsin, collagenase, elastase, pronase, dispase, DNase, and hyaluronidase, alone or in various combinations, [e.g., elastase and DNase for type II alveolar cell isolation (2002), collagenase with Dispase, and col-lagenase with hyaluronidase].

4. There are other, nonmammalian enzymes, such as Trypzean ,a recombinant, maize-derived, trypsin, TrypLE (Invitrogen), recombinant microbial, and Accutase and Accumax (Innovative Cell Technologies), also available for primary disaggregation. Crude preparations are often more successful than purified enzyme preparations, because the former contain other proteases as contaminants, although the latter are generally less toxic and more specific in their action.

5. Trypsin and pronase give the most complete disag-gregation, but may damage the cells. Collagenase and dispase, on the other hand, give incomplete disaggregation, but are less harmful. Hyaluronidase can be used in conjunction with collagenase to digest the intracellular matrix, and DNase is used to disperse DNA released from lysed cells; DNA tends to impair proteolysis and promote reaggregation.

6. Although each tissue may require a different set of conditions, certain requirements are shared by most of them:

    1. Fat and necrotic tissues are best removed during dissection.

    2. The tissue should be chopped finely with sharp instruments to cause minimum damage.

    3. Enzymes used for disaggregation should be removed subsequently by gentle centrifugation.

4. The concentration of cells in the primary culture should be much higher than that normally used for subculture, because the proportion of cells from the tissue that survives in primary culture may be quite low.

5. A rich medium, such as Ham's F12, is preferable to a simple medium, such as Eagle's MEM, and, if serum is required, fetal bovine often gives better survival than does calf or horse.

6. Isolation of specific cell types will probably require selective media. Embryonic tissue disaggregates more readily, yields more viable cells, and proliferates more rapidly in primary culture than does adult tissue.

7. PRIMARY CULTURE:

   Several techniques have been devised for the disaggregation of tissue isolated for primary culture. These techniques can be divided into:

   1. Mechanical disaggregation:

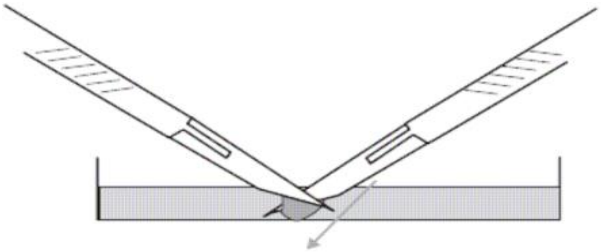
      1. For the disaggregation of soft tissues (e.g. spleen, brain, embryonic liver, soft tumors), mechanical technique is usually employed. This technique basically involves careful chopping or slicing of tissue into pieces and collection of spill out cells.

2. The cells can be collected by two ways:

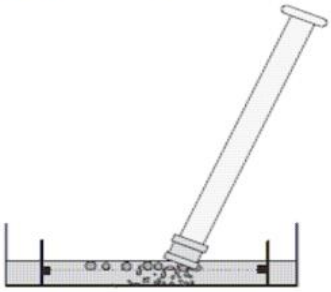
    1. Pressing the tissue pieces through a series of sieves with a gradual reduction in the mesh size.

    2. Forcing the tissue fragments through a syringe and needle.

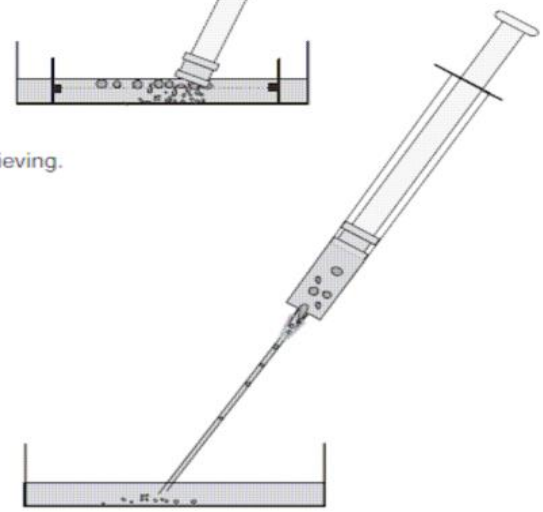
3. Although mechanical disaggregation involves the risk of cell damage, the procedure is less expensive, quick and simple. This technique is particularly useful when the availability of the tissue is in plenty, and the efficiency of the yield is not very crucial. It must however, be noted that the viability of cells obtained from mechanical techniques is much lower than the enzymatic technique.



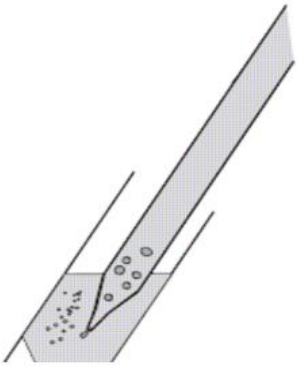
(a) Scraping or "spillage".



(b) Sieving.



(c) Syringing.



Mechanical Disaggregation

2. Enzymatic Disaggregation:

    1. Enzymatic disaggregation is mostly used when high recovery of cells is required from a tissue. Disaggregation of embryonic tissues is more efficient with higher yield of cells by use of enzymes. This is due to the presence of less fibrous connective tissue and extracellular matrix. Enzymatic disaggregation can be carried out by using trypsin, collagenase or some other enzymes.

    2. Disaggregation by trypsin:

        1. The term trypsinization is commonly used for disaggregation of tissues by the enzyme, trypsin.

        2. Many workers prefer to use crude trypsin rather than pure trypsin for the following reasons:

            1. The crude trypsin is more effective due to the presence of other proteases

            2. Cells can tolerate crude trypsin better.

            3. The residual activity of crude trypsin can be easily neutralized by the serum of the culture media (when serum-free media are used, a trypsin inhibitor can be used for neutralization).

3. Disaggregation of cells can also be carried out by using pure trypsin which is less toxic and more specific in its action. The desired tissue is chopped to 2-3 mm pieces and then subjected to disaggregation by trypsin.

4. There are two techniques of trypsinization-warm trypsinization and cold trypsinization.

    1. Warm trypsinization:

        1. This method is widely used for disaggregation of cells. The chopped tissue is washed with dissection basal salt solution (DBSS), and then transferred to a flask containing warm trypsin (37°C).

        2. The contents are stirred, and at an interval of every 30 minutes, the supernatant containing the dissociated cells can be collected.

        3. After removal of trypsin, the cells are dispersed in a suitable medium and preserved (by keeping the vial on ice). The process of addition of fresh trypsin (to the tissue pieces), incubation and

collection of dissociated cells (at 30 minutes intervals) is carried out for about 4 hours. The disaggregated cells are pooled, counted, appropriately diluted and then incubated.

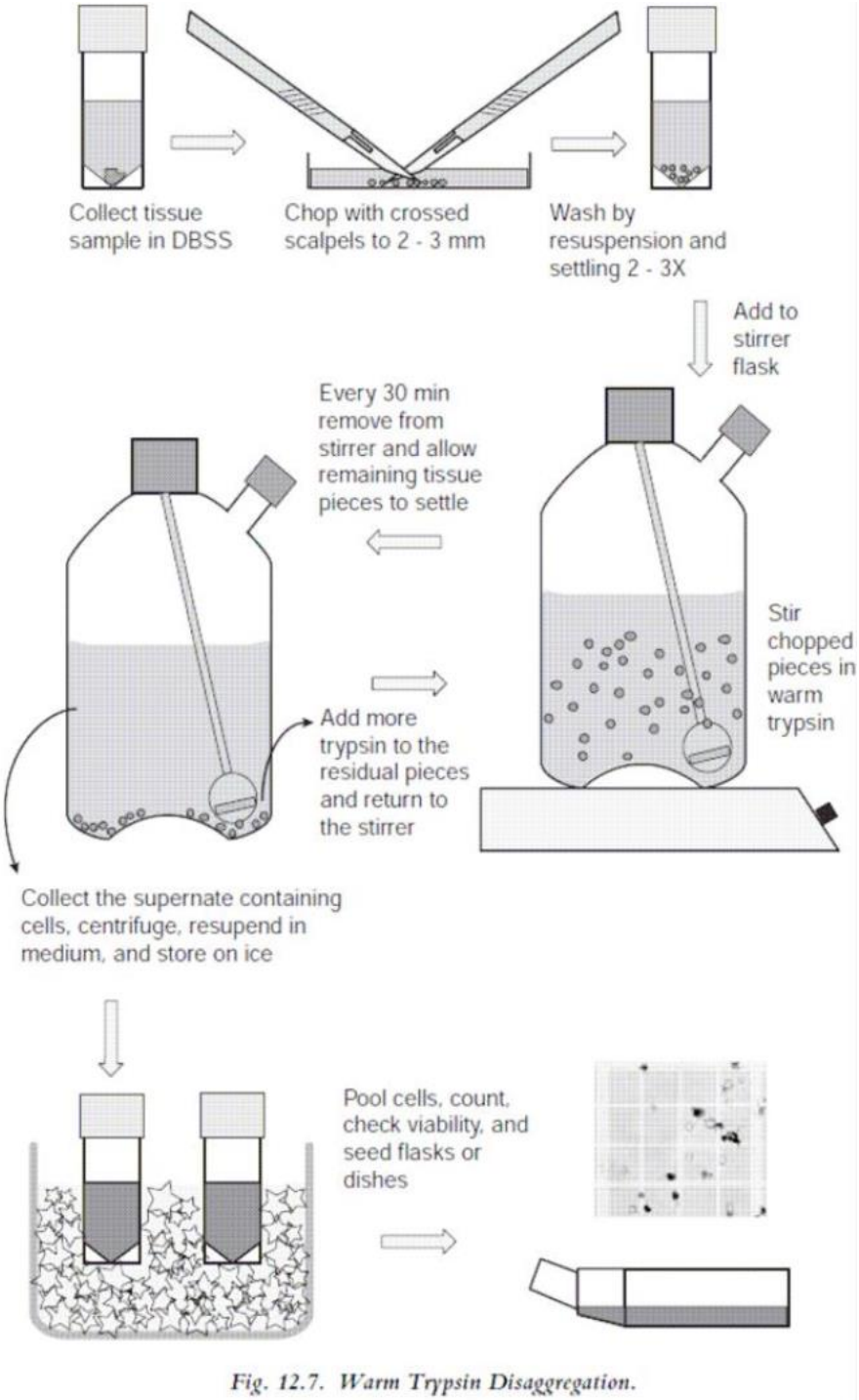


Fig. 12.7. Warm Trypsin Disaggregation.

Warm Trypsin Disaggregation

2. Cold trypsinization:

1. This technique is more appropriately referred to as trypsinization with cold pre-exposure. The risk of damage to the cells by prolonged exposure to trypsin at 37°C (in warm trypsinization) can be minimized in this technique.

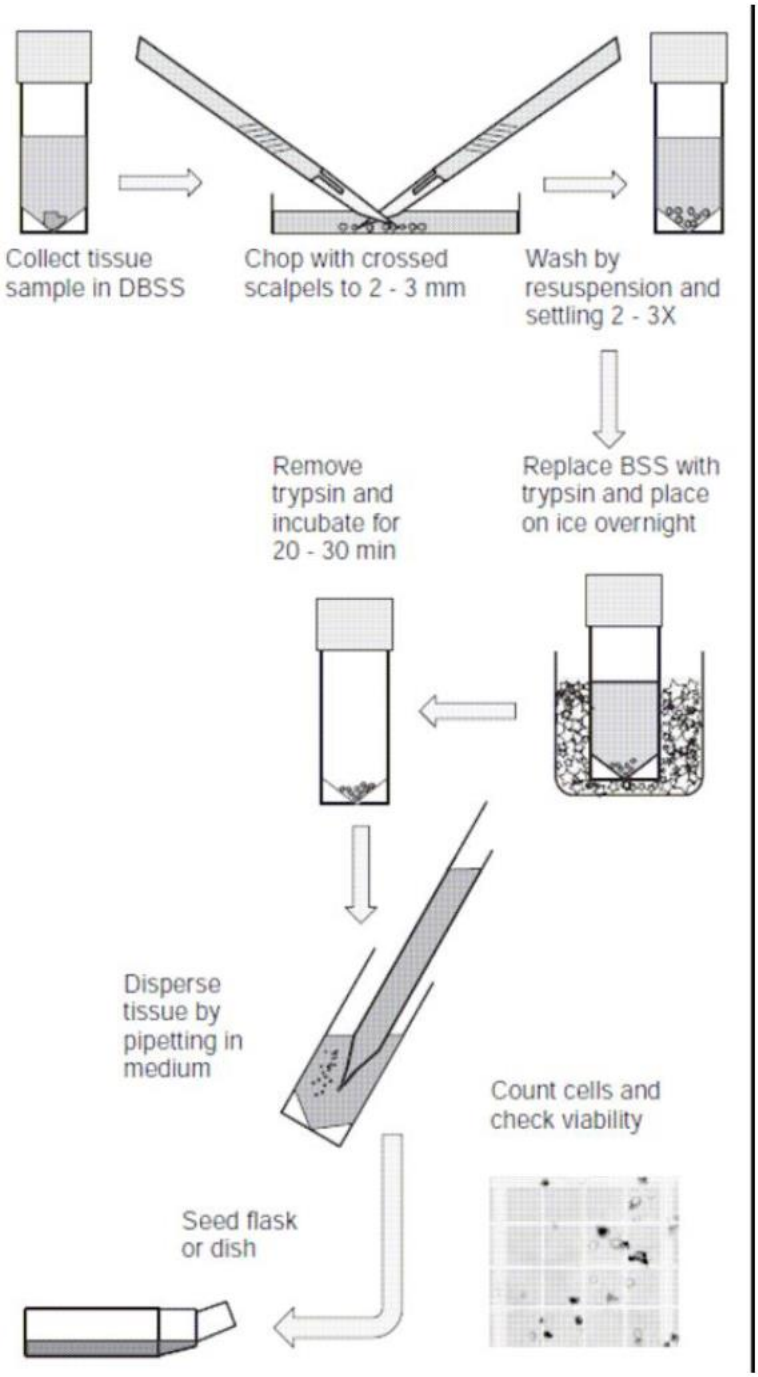
2. After chopping and washing, the tissue pieces are kept in a vial (on ice) and soaked with cold trypsin for about 6-24 hours. The trypsin is removed and discarded. However, the tissue pieces contain residual trypsin.

3. These tissue pieces in a medium are incubated at 37°C for 20-30 minutes. The cells get dispersed by repeated pipettings. The dissociated cells can be counted, appropriately diluted and then used.

4. The cold trypsinization method usually results in a higher yield of viable cells with an improved survival of cells after 24 hours of incubation. This method does not involve stirring or centrifugation, and

can be conveniently adopted in a laboratory. The major limitation of cold trypsinization is that it is not suitable for disaggregation of cells from large quantities of tissues.

5. Limitations of trypsin disaggregation: Disaggregation by trypsin may damage some cells (e.g. epithelial cells) or it may be almost ineffective for certain tissues (e.g. fibrous connective tissue). Hence other enzymes are also in use for dissociation of cells.



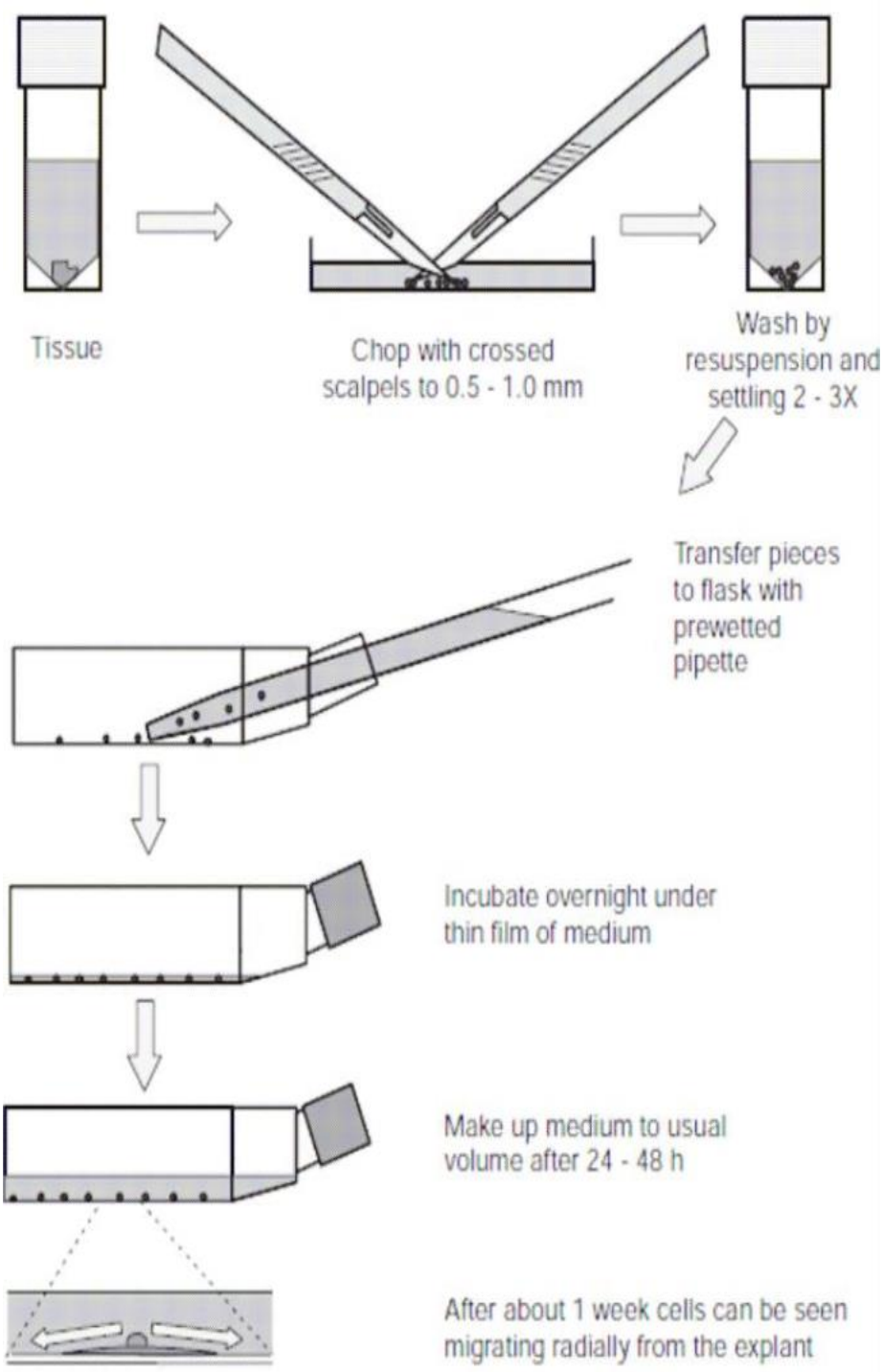
COLD TRYPSIN DISAGGREGATION

Cold Trypsin Disaggregation

3. Primary Explant Technique:

    1. The primary explant technique was, in fact the original method, developed by Harrison in 1907. This technique has undergone several modifications, and is still in use.

    2. The simplified procedure adopted for primary explant culture is depicted in Figure, and briefly described below.



Primary Explant Technique

3. The tissue in basal salt solution is finely chopped, and washed by settlings. The basal salt solution is then removed. The tissue pieces are spread evenly over the growth surface. After addition of appropriate medium, incubation is carried out for 3-5 days. Then the medium is changed at weekly intervals until a substantial outgrowth of cells is observed. Now, the explants are removed and transferred to a fresh culture vessel.

4. The primary explant technique is particularly useful for disaggregation of small quantities of tissues (e.g. skin biopsies). The other two techniques mechanical or enzymatic disaggregation, however, are not suitable for small amounts of tissues, as there is a risk of losing the cells.

5. The limitation of explant technique is the poor adhesiveness of certain tissues to the growth surface, and the selection of cells in the outgrowth. It is however, observed that the primary explant technique can be used for a majority of embryonic cells e.g. fibroblasts, myoblasts, epithelial cells, glial cells.

1.1.2 Maintenance and culture of Secondary Cell Lines:

1. This simply refers to the first passaging of cells, a switch to a different kind of culture system, or the first culture obtained from a primary culture. This is usually carried out when cells in adherent cultures occupy all the available substrate or when cells in suspension cultures surpass the capacity of the medium to support further growth, and cell proliferation begins to decrease or ceases completely. So as to maintain optimal cell density for continued growth and to encourage further proliferation, the primary culture has to be subcultured. This process is known as secondary cell culture.

2. **Cell line:** Once a primary culture is subcultured or passaged it represents a cell line. A cell line that experiences indefinite growth of cells during subsequent subculturing is called a continuous cell line, whereas finite cell lines experience the death of cells after several subcultures.

3. **Cell strain:** A cell line is a permanently established cell culture which will proliferate forever if a suitable fresh medium is provided continuously, whereas cell strains have been adapted to culture but, unlike cell lines, have a finite division potential. A cell strain is obtained either from a primary culture or a cell line. This is done by selection or cloning of those particular cells having specific properties or characteristics (e.g. specific function or karyotype) which must be defined.

Primary cell culture	Secondary cell culture
Directly obtained from animal or plant tissue.	Originates from a primary cell culture.
Closely resembles the parental tissue.	Does not closely resemble the parental tissue.
The biological response of the cell may be closer to that in an *in vivo* environment.	The biological response of the cell differs from that an *in vivo* environment.
The first culture derived from original cells/tissue (from an *in vivo* environment).	Derived from an existing culture.
Cannot be transformed.	Can be transformed.
Less chance of mutation.	Can increase the chance of mutation or genetic alteration of primary cells.
Acquired through steps of rinsing, dissection, and mechanical or enzymatic disaggregation.	If the primary culture is an adherent culture, the first step is to detach cells from the attachment (the surface of the culture vessel) by mechanical or enzymatic means. Then, the cells have to be detached from each other to form a single-cell suspension.
Finite life span.	Prolongs the life span of cells. Periodic subculturing may produce immortal cells through transformation or genetic alteration of primary cells.
The risk of contamination is high. More difficult to maintain.	The risk of contamination is lower. Comparatively easy to maintain.

1.2 Organ Culture

1. Organ culture is a development from tissue culture methods of research, the organ culture is able to accurately model functions of an organ in various states and conditions by the use of the actual in vitro organ itself.

2. Parts of an organ or a whole organ can be cultured in vitro. The main objective is to maintain the architecture of the tissue and direct it towards normal development. In this technique, it is essential that the tissue is never disrupted or damaged. It thus requires careful handling.

3. The media used for a growing organ culture are generally the same as those used for tissue culture. The techniques for organ culture can be classified into

    1. those employing a solid medium and
    2. those employing liquid medium

4. Methodology:

    Embryonic organ culture is an easier alternative to normal organ culture derived from adult animals. The following are four techniques employed for embryonic organ culture:

1. Plasma clot method:

    1. Prepare a plasma clot by mixing 15 drops of plasma with five drops of embryo extract in a watch glass.

    2. Place a watch glass on a pad of cotton wool in a petri dish; cotton wool is kept moist to prevent excessive evaporation from the dish.

    3. Place a small, carefully dissected piece of tissue on top of the plasma clots in watch glass.

    The technique has now been modified, and a raft of lens paper or rayon net is used on which the tissue is placed. Transfer of the tissue can then be achieved by raft easily. Excessive fluid is removed and the net with the tissue placed again on the fresh pool of medium.

2. Agar gel method:

    1. Media solidified with agar are also used for organ culture and these media consist of 7 parts 1% agar in BSS, 3 parts chick embryo extract and 3 parts of horse serum. Defined media with or without serum are also used with agar. The medium with agar provides the mechanical support for organ culture. It does

not liquefy. Embryonic organs generally grow well on agar, but adult organ culture will not survive on this medium.

2. The culture of adult organs or parts from adult animals is more difficult due to their greater requirement of oxygen. A variety of adult organs (e.g. the liver) have been cultured using special media with special apparatus (Towell's II culture chamber).

3. Since serum was found to be toxic, serum-free media were used, and the special apparatus permitted the use of 95% oxygen.

3. Raft Methods:

1. In this approach the explant is placed onto a raft of lens paper or rayon acetate, which is floated on serum in a watch glass. Rayon acetate rafts are made to float on the serum by treating their 4 corners with silicone.

2. Similarly, floatability of lens paper is enhanced by treating it with silicone. On each raft, 4 or more explants are usually placed.

3. In a combination of raft and clot techniques, the explants are first placed on a suitable raft, which is then kept on a plasma

clot. This modification makes media changes easy, and prevents the sinking of explants into liquefied plasma.

5. Application:

    1. The mitotic process and its modification by stimulants or suppressors have been studied in many cell types.

    2. Visible light has some inhibitory effects upon living cells. The lethal effects of X-irradiation can be quantified on mouse cells, and the effects of radiation upon cell constituents and upon DNA and RNA synthesis can be studied.

    3. Differentiation at the cellular level has mostly been studied in organ, rather than cell cultures.

    4. The uses of tissue culture in the study of cancer can be studied.

    5. Comparison of enzyme activities in cells in culture with those from the mouse have been made.

1.3 Cancer Cell Lines

1. Cancer cell lines are valuable in vitro model systems that are widely used in cancer research and drug discovery. Their use is primarily linked to their peculiar capability to provide an indefinite source of biological material for experimental purposes.

2. The establishment of a new cell line is a very complex process that is still not well understood. The success rate for the establishment is low and unpredictable for any specimen of origin. This statement could seem paradoxical when considering that the stabilization of a cell line starts with a sample of tumors able to grow vigorously in vivo, escaping all cellular mechanisms that are involved in the control of the cell cycle and cell death by apoptosis. However, many causes of this difficulty and serendipity for the establishment of a new cell line can be understood by taking into consideration the extreme differences (such as growth factor dependence, the percentage of oxygen, interaction with the stroma and immune cells, etc.) that exist between the in vivo and in vitro microenvironments.

3. This issue is witnessed by the impossibility of establishing, for example, a continuous cell line from chronic myeloid leukemia in the chronic phase. This hematological disorder is characterized by a very high rate of proliferation of leukemic cells in vivo, but the same leukemic cells die after a few weeks in vitro. Furthermore, regarding the success of continuous growth in vitro, the procedure for the establishment of a new cell line is, in any case, difficult and time consuming, requiring even more than one or two years. Nevertheless, since each cell line is derived from the disease from

which the patient is suffering from, its offers the opportunity for disclosing pathological features that were otherwise unidentified by conventional clinical diagnostic settings and to perform experiments that are not possible to be performed in vivo.

4. The processes of stabilizing and characterizing a new cell line should be performed in agreement with published guidelines. In particular, in 1999, Drexler and Matsuo published the “Guidelines for the characterization and publication of human malignant hematopoietic cell lines” and stressed the importance of confirming the immortality, authenticity, and tissue or cell type of origin for each newly established cell line.

5. These guidelines are still valid and they are included in the updated United Kingdom Coordinating Committee on Cancer Research (UKCCCR) guidelines for the use of cancer cell lines in biomedical research published by Geraghty et al. in 2014. Indeed, a detailed characterization, the immortality of the culture, a proof of neoplasticity, authentication of the true origin of the cells, scientific significance and availability of the cell line for other investigators are of paramount importance when publishing a new cell line. In this way, under the right conditions and with appropriate controls, properly authenticated cancer cell lines retain most of the properties of the cancer of origin and they become helpful model systems for the progress of medical research.

6. Recent findings that were obtained by the characterization of hundreds of cell lines with omics technologies (i.e., genomics, transcriptomics, and proteomics) reinforced the concept of cell line usefulness in medical

research. Indeed, for the majority of the existing cancer cell lines, these data have been published and made available through online datasets, which made it possible to explore detailed molecular and cellular alterations, such as mutations, copy number variations, and gene and protein expression profiles, featuring each cell line. In this way, the process for selecting the most appropriate model systems for experimental purposes has been significantly enhanced.

1.4 Tissue Engineering

1. Skin:

    1. Engineering skin tissue is mainly focused on the restoration of dermis and epidermis layers rather than the regeneration of skin appendages (such as hair, nail and glands). Various strategies have been attempted to re-establish the native skin without compromising its functions. Bioengineers have aimed to enhance the wound healing process in the native skin tissue by delivering drugs such as antibiotics to control infection and growth factors/genes to speed up the re-epithelialisation, angiogenesis and/or granulation tissue phases (Andreadis and Geer, 2006).

    2. Other researchers may choose to regenerate the epidermal or both epidermal and dermal layers by implanting acellular or cellular matrix. Moreover, by surface modification of the scaffold, researchers

have improved the biological properties of tissue engineered scaffolds such as cell adhesion, proliferation, viability and differentiation.

3. Complete restoration of functional skin tissue chiefly depends upon the vital determinants such as the choice of biomaterial, scaffold fabrication methodology, cell lines and surface topography such as pores, ridges, grooves, fibres, nodes, and so on (Subramanian et al, 2009). Tissue engineering skin remains a major scientific and clinical challenge. Wound healing can be categorised into distinct phases and the stages of healing. The processes are controlled using a series of cellular and biochemical responses.

4. Skin tissue engineering is a rapidly developing field based on advances made in the last quarter of the twentieth century, both in cellular culture technique and in biocompatible matrix technology. Materials can be devoid of cells and used in vivo to guide fibroblast invasion, collagen deposition, and neodermal production. The materials in this group are invariably bilayers, all consisting of an integrating dermal component, and a temporary epidermal component designed merely to prevent evaporative water loss and signal wound “closure.” The second group of materials are usually monolayer scaffolds into which bilayer composite cultured skins have been created in vitro.

5. Skin tissue engineering was one of the early organ systems to which regenerative medicine techniques were applied, often in situations when autologous skin grafting is insufficient or not available. As a result, engineered dermal tissue could be the key to providing sufficient healthy donor skin for engraftment for patients with large burn surface areas. Additionally, the ability of fetal cells to stimulate skin regeneration has also been investigated.

6. The majority of current research in skin tissue engineering focuses on the synthesis of complex three-dimensional (3D) polymer scaffolds containing functional biomolecules to which cells are introduced, leading to scaffold/skin constructs for regeneration. Hohlfeld and associates developed fetal skin constructs to improve healing of severe burns (Hohlfeld et al., 2005). Their simple techniques provided complete treatment without traditional skin grafting, showing that fetal skin cells might have great potential to treat burns and eventually acute and chronic wounds of other types.

7. Sun et al. showed that co culture with fibroblasts enables keratinocytes and endothelial cells to proliferate without serum, and that keratinocytes and endothelial cells appear to self-organize according to the native epidermal-dermal structure given the symmetry-breaking field of an air-liquid interface (Sun et al., 2005). Kaviani et al. consistently isolated subpopulations of fetal mesenchymal cells from human amniotic fluid and rapidly expanded them in vitro (Kaviani et al., 2003). These human mesenchymal amniocytes attach firmly to both polyglycolic

acid polymer and acellular human dermis, and thus it was hypothesized that amniotic fluid may be a valuable and practical cell source for fetal tissue engineering.

8. Another potential source for skin tissue engineering is human umbilical cord-derived stem cells. Preliminary reports have described the use of cord blood cells combined with a fibrin/platelet glue for treatments of human skin wounds, with significant subjective improvement (Valbonesi et al., 2004). Importantly, no evidence of a host immune response was seen.

9. Several other studies have demonstrated successful growth of many of the complex components of human skin from umbilical cord blood, including small blood vessels (Wu et al., 2004). Further studies into the use of this and other types of fetal tissue, both for engineering of skin replacements and for acceleration of wound healing, are ongoing.

2. Liver:

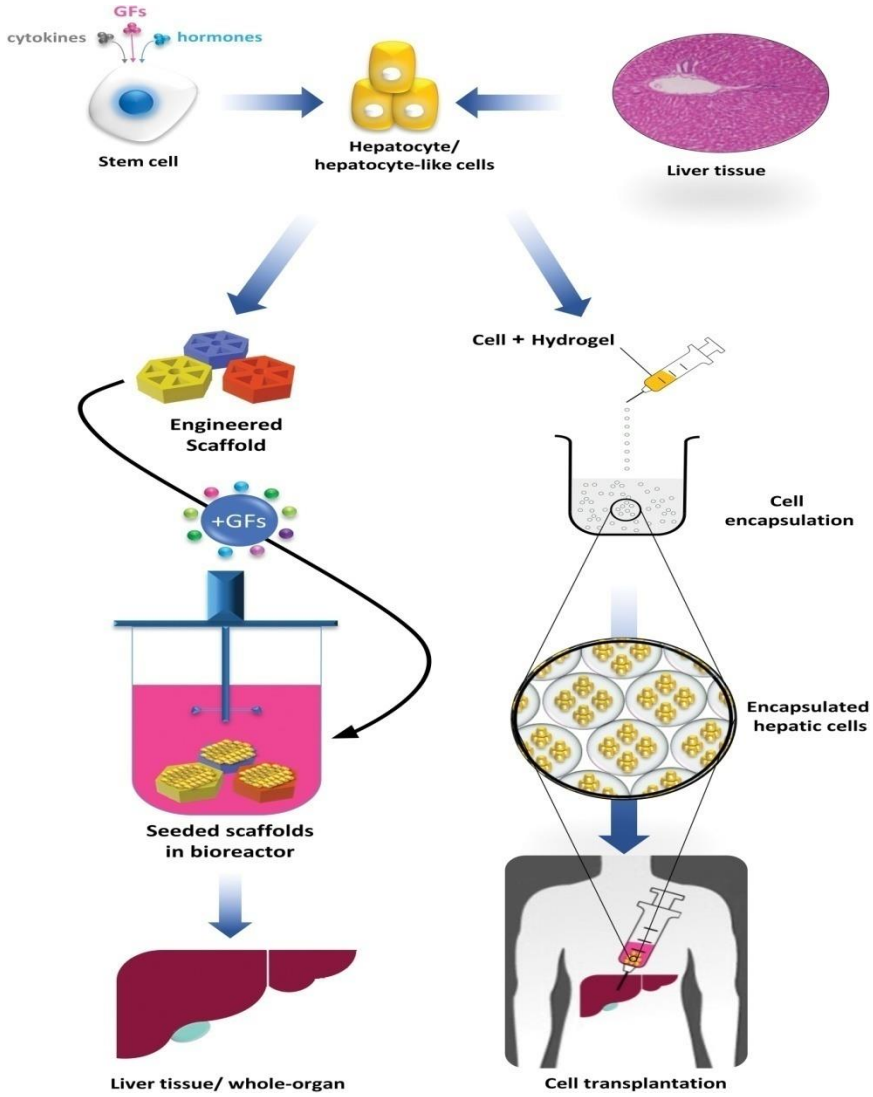
1. Every year, millions of people face liver disorders, including chronic and acute liver failure. As the largest organ in the body, the liver undertakes various responsibilities, having an essential role in health and lifespan due to its metabolic functions like protein production, bile production, and drug detoxification. There are a few therapeutic approaches for liver dysfunction; that is why many liver donors have been demanded. Due to the short supply of liver transplants,

difficulties with the immunosuppressive drugs, and the possibility of transplanted tissue rejection, liver tissue engineering (LTE) basis seems to be an alternative.

2. In recent years, many types of research have targeted the unique self-regeneration characteristic of the liver. Cell therapy demonstrates meaningful results, even though the low efficiency is a significant disadvantage. Other approaches, such as dialysis, have also been used for short-term survival in patients and drug metabolism and blood filtration of the liver. However, these methods are not sufficient for significant liver failure and can only be used for short-time applications.

3. The functionality of HCs (hepatocyte cells) should be enhanced through external intervention using regenerative medicine. Cell encapsulation has been widely used as a bridge approach. The bridge approach protects the cells using a membrane or capsule to increase the cellular proliferation rate and cell viability until liver transplantation is available. In acute liver failure, there is a lack of function; this approach gives time for regeneration and remodeling of the liver by itself, by retrieving some of liver functions.

4. The term LTE is one of the main goals in biotechnology: the possibility of the full or partial liver's function retrieval as a treatment for chronic and acute liver failure and eventually generating an entire functional organ for transplantation or as an extracorporeal device. Most technical approaches in LTE are based on HCs or hepatocyte-like cells (HLCs) seeded on a three-dimensional (3D) scaffold, which can maintain the viability and functional phenotype of the cells.



Overview of the LTE. HCs/HLCs obtained from stem cells or natural liver tissue are used in two main LTE approaches (scaffold based and encapsulation based). In scaffold-based mode, cells are utilized for seeding on engineered scaffolds to ideally attain a liver tissue/whole organ. In the encapsulation-based approach, cells are encapsulated for more efficient cell transplantation applications. Each of these approaches is directed with specific signaling molecules. HCs, hepatocyte cells; HLCs, hepatocyte-like cells; LTE, liver tissue engineering.

5. Various cells can be utilized in LTE from different sources, and each has its advantages. According to the application and purpose of the study, stem cells (SCs), primary human or animal liver cells, are used. As the supporting substrate, various scaffolds with both natural and synthetic materials have been manufactured with adaptable fabrication methods, including conventional and rapid prototyping (RP) techniques. Parameters affecting the function of scaffolds and, therefore, cell behavior have been studied. Chemical, morphological, mechanical, and geometrical cues can alter this behavior, which is described as the material used, porosity and pore size, biomechanical properties, and scaffold design, respectively.

6. However, the stability of hepatic phenotype and proliferation capacity along with the main liver functions have not yet arrived at a satisfying value. Up to now, investigations toward therapeutic liver methods with engineering insight have not been widely carried out because of the liver's natural complexity and its essential role in vitality. Therefore, for putting together the best parameters to achieve better results, it is necessary to review previous works.

7. Human primary hepatocytes (hPHs):

    1. hPHs are one of the most matured liver cell sources; while they comprise 60% of the liver volume and have a high proliferation capacity in vivo, in vitro utilization of these cells leads to losing their specified functions, which is a cause of considerable difference between their native environment and the laboratory simulations. As a result, they dedifferentiate in traditional cell cultures. However, researches show that the function and viability of these cells enhance when they are in the form of aggregations. In this regard, improvements are needed to optimize the size of aggregates to prevent necrosis in the center and encourage forming connections between aggregations for facilitated nutrition, oxygen transportation, and vessel formation. Their limitations are the low capacity of growth and phenotype instability in vitro, as well as a complicated isolation procedure.

    2. An approach that had promising results to overcome these limitations is coculturing hPHs with other nonparenchymal cells (NPCs) such as sinusoidal endothelial and stellate cells and fibroblasts to mimic their microenvironment.

8. Animal primary hepatocytes (aPHs):

    1. Animal cells are famous for their availability, but because of their limited biocompatibility, they should be used under some special conditions, and one of the approaches that is a desirable match for animal cells is cell encapsulation.

    2. Two kinds of animal primary hepatocytes have been used in liver regenerative medicine:

        1. Rat hepatocytes:

            1. Primary rat hepatocytes have been used widely in cell encapsulation and in some cases with scaffolds. The first step to getting primary rat hepatocytes is isolation, that is, one of the limitations for animal cells due to the hard isolation procedure and the low viability of the cells needed in TE approaches. Despite this step, there are many advantages in using primary rat hepatocytes that will be discussed. In cell encapsulation, adult Sprague Dawley rats and Wistar rats are usually used. In some studies, these cells are utilized with other cells and growth factors.

2. For instance, primary rat hepatocytes were encapsulated with Fetal liver cell (FLC) and β-fibroblast growth factor (β-FGF), and it was shown that the function of this kind of rat hepatocytes was increased during 7 days in vitro tests as a result of FLC and β-FGF incorporation. In another study, hPHs and primary rat hepatocytes were compared, while encapsulating in alginate hydrogel, and it was shown that these two types had the same viability in a specific time, but rat hepatocytes showed better cell functions such as albumin and ALT secretion.

2. Porcine hepatocytes:

    1. Another type of animal cells that is used worldwide for LTE is porcine hepatocytes, due to its availability and the fact that its functions are closer to human liver tissue. Another advantage of these cells is the level of ethics issue, which is much lower than human types and are various due to the animal used. An instance in co-culture systems, many kinds of these cells can be utilized even with other human cells to show the influence of human cells or also to model diseases like cancers.

3. Pancreas:

    1. Diabetes mellitus (DM) due to loss of insulin-secreting ß-cells, because of either autoimmune processes in type I DM or surgical resection of the pancreas, represents a suitable model for cell-based therapies. Although the current gold standard for the management of DM is exogenous insulin therapy in response to elevated blood glucose levels, this treatment option is inferior to continuous endogenous insulin secretion by ß-cells. Therefore, alternative therapies are needed that restore insulin-secreting function and avoid adverse effects such as recurrent hypoglycemia and long-term complications.

    2. An alternative for patients refractory to exogenous insulin injection is islet transplantation following the Edmonton protocol. The Edmonton protocol is a state-of-the-art procedure that comprises clinical isolation of human islet cells from cadaveric donors, purification of the islets after digestion, intraportal transplantation, and a glucocorticoid-free immunosuppressive regimen for the recipient after transplantation.

    3. Despite improvements in the isolation and cell culture protocol and use of various implantation sites for the ß-cells, only 60%–85% of the patients are independent of insulin at 1 year after transplantation, and this figure decreases with the passage of time. Fewer than 20% of the patients remain insulin-independent for 5 years. The reasons for apoptosis of the transplanted allogenic islets and failure of this

treatment include non-immune-related, instant blood-mediated inflammatory reactions (IBMIR), graft–host reactions, and a lack of engraftment due to insufficient oxygen supply and increased levels of toxins or pharmaceuticals at the intraportal or intrahepatic transplantation site, respectively.

4. Another limiting factor is the global shortage of suitable donor organs. Together, these findings show the need for improvement in techniques for restoration of insulin-secreting function. The tissue engineering approaches reviewed here are intended to overcome the current limitations.

5. In the emerging field of tissue engineering, scaffolds replace the extracellular matrix (ECM) with the intention of mimicking native tissues to provide an optimal environment for cells. Scaffolds, cells, and growth-stimulating factors, often referred to as the tissue engineering triad, are essential to create bioartificial organs. In native tissues, the ECM contributes to viability and function of cells by:

    1. providing structural support
    2. ensuring mechanical stability
    3. regulating cellular activities
    4. storing and releasing growth factors
    5. providing a degradable environment that can be remodeled on demand

6. To replicate each of these ECM functions, biological and synthetic materials such as porous scaffolds for cell seeding, decellularized ECM (dECM) scaffolds, scaffold cell sheets, and cell-encapsulating hydrogels are currently used to host insulin-secreting cells (ISC). The application of scaffolds ranges from the generation of ISC from stem cells and progenitor cells in scaffold-based three dimensional (3D) culture systems to the building of artificially created support systems that serve as a logistic template.

7. These support systems are intended to prevent anoikis, protect the patient from inflammatory and immunological host reactions, and improve long-term viability. The use of scaffold-based tissue engineering in routine treatment of these patients is still hampered by:

    1. reduced vascularization and consequential insufficient supply and hypoxia of the containing cells

    2. immunological host–graft reactions against cells and scaffolds

    3. a lack of scaffolding techniques that precisely mimic nature

1.5 Assisted Reproductive Technology

1. Assisted reproductive technology (ART) is the technology used to achieve pregnancy in procedures such as fertility medication, artificial insemination, in vitro fertilization and surrogacy. It is reproductive technology used primarily for infertility treatments, and is also known as fertility treatment.

2. With ART, the process of sexual intercourse is bypassed either by artificial insemination or fertilization of the oocytes in the laboratory environment (i.e., in vitro fertilization). In general, ART procedures involve surgically removing eggs from a woman's ovaries, combining them with sperm in the laboratory, and returning them to the woman's body or donating them to another woman." According to CDC, "they do not include treatments in which only sperm are handled (i.e., intrauterine—or artificial—insemination) or procedures in which a woman takes medicine only to stimulate egg production without the intention of having eggs retrieved.

3. Procedures are mainly fertility medication, as well as ART techniques that use more substantial and forceful interventions, of which in vitro fertilization (IVF) and expansions of it (e.g. OCR, AZH, ICSI, ZIFT) are the most prevalent. However, there are also other manual ART, not necessarily dependent on IVF (e.g. PGD, GIFT, SSR):

    1. Fertility medication: Most fertility medications are agents that stimulate the development of follicles in the ovary. Examples are gonadotropins and gonadotropin releasing hormone.

2. Artificial insemination: Artificial insemination involves sperm being placed into a female's uterus (intrauterine) or cervix (intracervical) using artificial means rather than by sexual intercourse. This can be a very low-tech process, performed at home by the woman alone or with her partner.

3. In-vitro Fertilization: In vitro fertilization is the technique of letting fertilization of the male and female gametes (sperm and egg) occur outside the female body. Techniques usually used in in vitro fertilization include:

    1. Transvaginal ovum retrieval (OCR) is the process whereby a small needle is inserted through the back of the vagina and guided via ultrasound into the ovarian follicles to collect the fluid that contains the eggs.

    2. Embryo transfer is the step in the process whereby one or several embryos are placed into the uterus of the female with the intent to establish a pregnancy.

    3. Assisted zona hatching (AZH) is performed shortly before the embryo is transferred to the uterus. A small opening is made in the outer layer surrounding the egg in order to help the embryo hatch out and aid in the implantation process of the growing embryo.

4. Intracytoplasmic sperm injection (ICSI):

    1. Intracytoplasmic sperm injection (ICSI) is beneficial in the case of male factor infertility where sperm counts are very low or failed fertilization occurred with previous IVF attempt(s). The ICSI procedure involves a single sperm carefully injected into the center of an egg using a microneedle. With ICSI, only one sperm per egg is needed. Without ICSI, you need between 50,000 and 100,000. This method is also sometimes employed when donor sperm is used.

        1. Autologous endometrial coculture is a possible treatment for patients who have failed previous IVF attempts or who have poor embryo quality. The patient's fertilized eggs are placed on top of a layer of cells from the patient's own uterine lining, creating a more natural environment for embryo development.

        2. In zygote intrafallopian transfer (ZIFT), egg cells are removed from the woman's ovaries and fertilized in the laboratory; the resulting zygote is then placed into the fallopian tube.

        3. Cytoplasmic transfer is the technique in which the contents of a fertile egg from a donor are injected into the infertile egg of the patient along with the sperm.

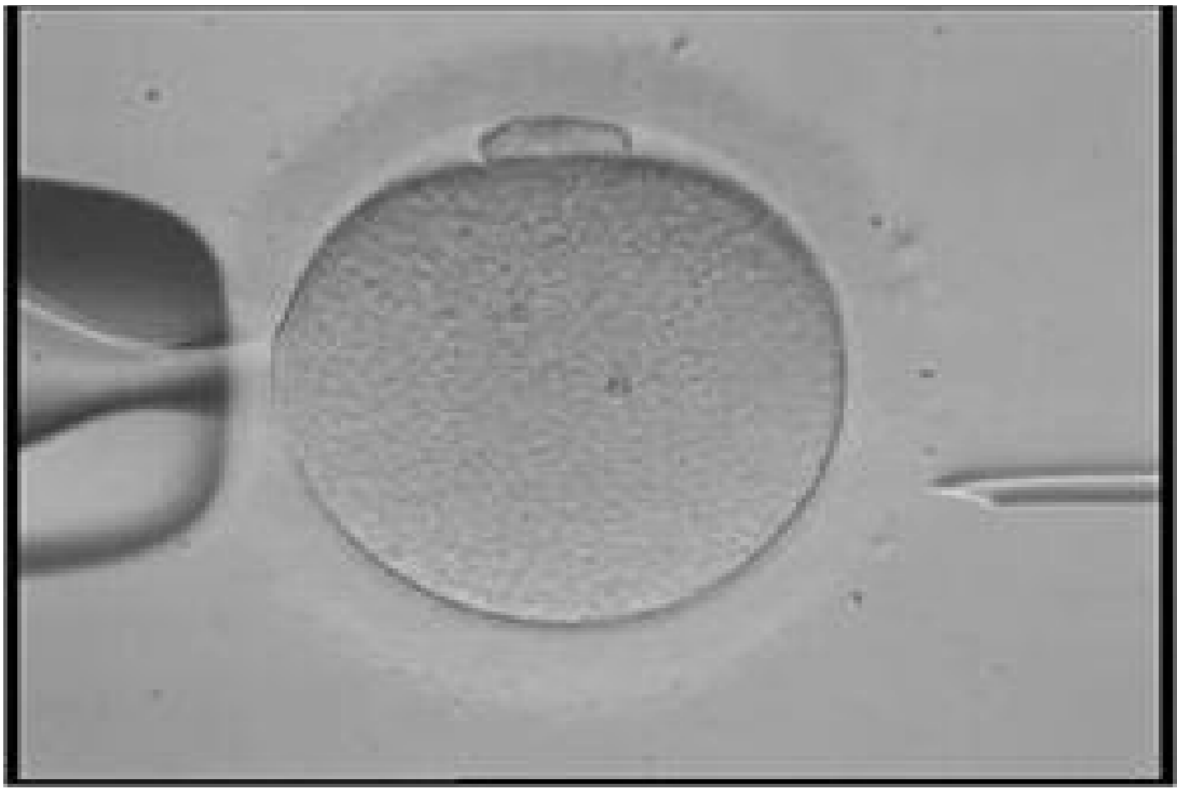
4. Egg donors are resources for women with no eggs due to surgery, chemotherapy, or genetic causes; or with poor egg quality, previously unsuccessful IVF cycles or advanced maternal age.

5. In the egg donor process, eggs are retrieved from a donor's ovaries, fertilized in the laboratory with the sperm from the recipient's partner, and the resulting healthy embryos are returned to the recipient's uterus.

6. Sperm donation may provide the source for the sperm used in IVF procedures where the male partner produces no sperm or has an inheritable disease, or where the woman being treated has no male partner.

7. Pre-implantation genetic diagnosis (PGD) involves the use of genetic screening mechanisms such as fluorescent in-situ hybridization (FISH) or comparative genomic hybridization (CGH) to help identify genetically abnormal embryos and improve healthy outcomes.

8. Embryo splitting can be used for twinning to increase the number of available embryos.



Intracytoplasmic sperm injection (ICSI)

Intracytoplasmic Sperm Injection

5. Surrogacy:

    1. Surrogacy, where a woman agrees to become pregnant and deliver a child for a contracted party. It may be her own genetic child, or a child conceived through natural insemination, in vitro fertilization or embryo transfer using another woman's ova. Surrogacy via a gestational carrier is an option when a patient's medical condition prevents a safe pregnancy, when a patient has ovaries but no uterus due to congenital absence or previous surgical removal, and where a patient has no ovaries and is also unable to carry a pregnancy to full term.

6. Embryo Transfer:

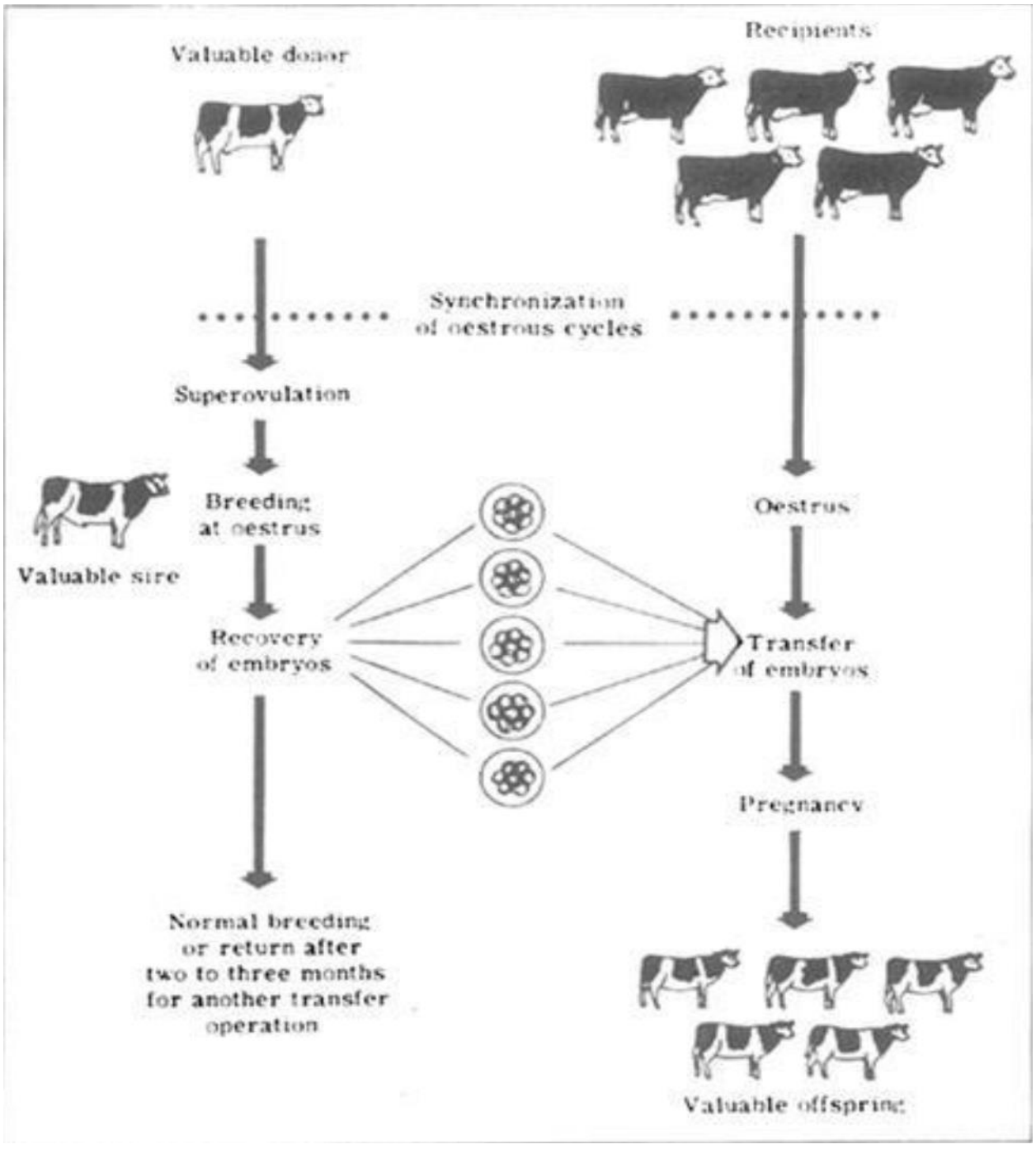
    1. Embryo transfer refers to a step in the process of assisted reproduction in which embryos are placed into the uterus of a female with the intent to establish a pregnancy. This technique [which is often used in connection with in vitro fertilization (IVF)], may be used in humans or in animals, in which situations the goals may vary.

    2. The donor cow is stimulated with a hormone (generally pregnant mare's serum gonadotrophin (PMSG) or follicle-stimulating hormone). Following such stimulation the animal comes on heat and she is bred or artificially inseminated with semen of the breeder's choice.

3. In order to obtain maximum fertility two breedings are generally employed with a 12–hour interval, and a higher dose of semen is used the second time. It is expected that the donor will release a number of ova (10 to 15 or more) at this oestrus instead of the usual one.

4. The ova are naturally fertilized within the donor and proceed to develop. If these embryos are left to grow in the donor cow, resorption and or multiple pregnancies may result which are undesirable in cattle because of resulting complications and the possible occurrence of freemartins.

5. About 4 to 5 days after breeding (when the embryos are freely “floating” within the uterus and are not yet implanted), the embryos are flushed out with a suitable biological medium. Embryos are transferred within four to five hours of recovery; during this time they are stored with minimum changes of temperature and pH. It is important that the oestrous cycle of the recipient animal be synchronous with that of the donor (both donor and recipients should be in heat on the same day).



Embryo Transfer

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SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY

UNIT – II - Medical Biotechnology – SBB3101

Subject Name: Medical Biotechnology Subject Code: SBB3101

SBB3101	MEDICAL BIOTECHNOLOGY	L	T	P	Credits	Total Marks
		4	0	0	4	100

COURSE OBJECTIVES

- To study about the medicinal approach of Biotechnology and recent advancements in diagnosis.

UNIT 1 ANIMAL CELL CULTURE
12 Hrs.

Animal cell culture-media, maintenance and culture of primary, secondary and continuous cell lines- organ culture-applications- cancer cell lines- apoptosis. Tissue Engineering – Skin, Liver, Pancreas. Assisted reproductive technology- Pregnancy diagnosis.

UNIT 2 CHROMOSOMAL ABNORMALITIES
12 Hrs.

Chromosomal disorders – Gene controlled diseases –Identification of disease genes Haemophilia, DMD, Alzheimer's – Molecular basis of human diseases: Pathogenic mutations – Oncogenes – Loss of function – Tumour Suppressor Genes Immunopathology: Hepatitis, Autoimmune Disorders.

UNIT 3 DIAGNOSTIC TECHNIQUES **12 Hrs.**

Prenatal diagnosis – Invasive techniques and Non-invasive techniques – Diagnosis of pathogenic microbes: Classical and modern methods- Diagnosis using protein and enzyme markers, DNA/RNA based diagnosis – Molecular markers – Microarray technology – genomic and cDNA arrays.

UNIT 4 PREVENTION AND TREATMENT **12 Hrs.**

Vaccines-conventional, recombinant, synthetic peptide, anti-idiotype, DNA vaccines Deletion mutant and vaccinia vector vaccine- Antibiotics-mode of action- antibacterial, antifungal, antiviral, antitumor antibiotics- synthetic chemotherapeutic agent development of microbial resistance to antibiotics.

UNIT 5 MODERN MEDICINE **12 Hrs.**

Hybridoma technique for MCAb production and applications- Gene therapy: Exvivo, In vivo, In situ- Cell and tissue engineering- Stem cell therapy- Nanomedicines- Gene products in medicine – Humulin, Erythropoietin, Growth Hormone/Somatostatin, tPA, Interferon.

Max Hours.60

TEXT / REFERENCE BOOKS:

1. Ramasamy, P. "Trends in Biotechnology", University of Madras, Pearl press, 2002. 2. Trevan. "Biotechnology". Tata McGraw-Hill, 2005.
2. Betty Forbes, Danial SAHM Alics Weinfield, Bailey 2007. Scott's diagnostic microbiology, 12th edition Mosby.
3. Jogdand, S. N. Medical Biotechnology, Himalaya Publishing house, Mumbai, 2005. 2. Click, B. R. and Pasternak. Molecular Biotechnology: Principle and applications of recombinant DNA. ASM Press, 2010.

END SEMESTER EXAMINATION QUESTION PAPER PATTERN

Max. Marks: 100 **Exam Duration: 3 Hrs.**
PART A: 10 questions of 2 marks each - No choice **20 Marks**
PART B: 2 questions from each unit of internal choice; each carrying 16 marks **80 Marks**

Unit 2: Chromosomal Abnormalities

AUTOSOMAL ABNORMALITIES

AUTOSOME RELATED CHROMOSOMAL ABNORMALITIES

TRISOMY 21- (DOWN'S SYNDROME)

Down's Syndrome English physician John Langdon Down first characterized Down syndrome as a separate form of mental disability in 1862.

• Genetic disorder caused by the presence of all or part of a third copy of chromosome 21.

• Most common chromosomal abnormality in humans, occurring in about 1 per 1000 babies born each year.

Signs And Symptoms

Characteristics Percentage: Mental impairment 99% Abnormal teeth 60% Stunted growth 90% Slanted eyes 60% Umbilical hernia 90% Shortened hands 60% Increased skin back of neck 80% Short neck 60% Low muscle tone 80% Obstructive sleep apnea 60% Narrow roof of mouth 76% Bent fifth finger tip 57% Flat head 75% Brushfield spots in the iris 56% Flexible ligaments 75% Single transverse palmar crease 53% Large tongue 75% Protruding tongue 47% Abnormal outer ears 70% Congenital heart disease 40% Flattened nose 68% Starbismus ~35% Separation of 1st and 2nd toes 68% Undescended testicles 20%

Brushfield spots, visible in the irises of a baby with Down syndrome.

Neurology • Have mild (IQ: 50–70) or moderate (IQ: 35– 50) intellectual disability with some cases having severe (IQ: 20–35) difficulties. • As they age, they perform less well compared to their same-age peers. • After 30 years of age may lose their ability to speak. • Causes about a third of cases of intellectual disability. • Developmental milestones are delayed with the ability to crawl typically occurring around 8 months rather than 5 months and the ability to walk independently typically occurring around 21 months rather than 14 months. • Better language understanding than ability to speak. • Between 10-45% have either a stutter or rapid irregular speech. • Behavior problems are not generally as great an issue as in other syndromes associated with intellectual disability. • Mental illness occurs in nearly

30% with autism occurring in 5–10%. • Wide range of emotions; generally happy, symptoms of depression and anxiety may develop in early adulthood.

Fertility • Males usually do not father children. • Females have lower rates of fertility relative those who are unaffected. • Menopause typically occurs at an earlier age. • As of 2006, there have been three recorded instances of males with Down syndrome fathering children and 26 cases of women having children. • Without assisted reproductive technologies, approximately half of the pregnancies of someone with Down syndrome will also have the syndrome.

Genetics • Down syndrome is caused by having three copies of the genes on chromosome 21, rather than the usual two. • Those who have one child with Down syndrome have about a 1% risk of having a second child with the syndrome, if both parents are found to have normal karyotypes.

Karyotype

• Trisomy 21 is caused by a failure of the 21st chromosome to separate during egg or sperm development. • About 88% of cases of trisomy 21 result from non separation of the chromosomes in the mother, 8% from non-separation in the father, and 3% after the egg and sperm have merged.

Screening Guidelines recommend that screening for Down's syndrome be offered to all pregnant women, regardless of age. Screen Week of pregnancy when performed Detection rate False positive Combined test 10–13.5 wks 82–87% 5% Quad screen 15–20 wks 81% 5% Integrated test 15–20 wks 94–96% 5% Cell-free fetal DNA From 10 wks 96-100% 0.3%

Ultrasound of a fetus with down's syndrome having a large bladder.

Prognosis • Have a higher risk of early death than the general population; often from heart problems or infections. • Currently between 4 and 12% die in the first year of life. • In those without heart problems 85% survive to 10 years and 80% survive to 30 years of age. • About 10% live to 70 years of age.

TRISOMY 13 (PATAU SYNDROME)

• Some or all of the cells of the body contain extra genetic material from chromosome 13. This can occur either because each cell contains a full extra copy of chromosome 13 (a disorder known as trisomy 13 or trisomy D), or because each cell contains an extra partial

copy of the chromosome (i.e., Robertsonian translocation) or because of mosaic Patau syndrome.

Trisomy 13 was first observed by Thomas Bartholin in 1657, but the chromosomal nature of the disease was ascertained by Dr. Klaus Patau in 1960.

• A partial trisomy - Part of chromosome 13 becomes attached to another chromosome in a Robertsonian translocation. • Not inherited, but occur as random events during the formation of reproductive cells. • Balanced translocation

Ultrasound (after 14 weeks) Normal baby Trisomy 13

KARYOTYPE ABNORMALITIES

NERVOUS SYSTEM RELATED- • Microcephaly • Holoprosencephaly (failure of the forebrain to divide properly). • Intellectual disability and motor disorder • Structural eye defects • Meningomyelocele (a spinal defect)

Musculoskeletal and Cutaneous •Proboscis •Low-set ears •Prominent heel •Deformed feet known as rocker-bottom feet •Abnormal palm pattern •Overlapping of fingers over thumb •Cutis aplasia •Cleft palate

Urogenital • Abnormal genitalia • Kidney defects • Other • Heart defects • Dextrocardia • Single umbilical artery

Treatment • Case-by-case basis and depends on the individual circumstances of the patient. • Surgery may be necessary to repair heart defects or cleft lip and cleft palate. • Physical, occupational, and speech therapy will help individuals with Patau syndrome reach their full developmental potential.

Prognosis • More than 80% of children with Patau syndrome die within the first year of life. • Children with the mosaic variation are usually affected to a lesser extent. • Patau syndrome affects somewhere between 1 in 10,000 and 1 in 21,700 live births.

TRISOMY 18 – (EDWARD'S SYNDROME)

Edward's Syndrome. Named after John Hilton Edwards, who first described the syndrome in 1960.

• A chromosomal disorder caused by the presence of all, or part of, an extra 18th chromosome. • Second-most common autosomal trisomy, after Down's syndrome, that carries to term.

• One in 6,000 live births, since many of the fetuses with the syndrome die before birth. • Very low rate of survival, resulting from heart abnormalities, kidney malformations, and other internal organ disorders.

• Has a lot to do with the mother's age just like down's syndrome. • Average maternal age for conceiving a child with this disorder is 32½.

Signs and symptoms

• Kidney malformations. • Structural heart defects at birth (i.e., ventricular septal defect, atrial septal defect, patent ductus arteriosus). • Intestines protruding outside the body (omphalocele), esophagial atresia, intellectual disability, developmental delays, growth deficiency, feeding difficulties, breathing difficulties. • Arthrogryposis (a muscle disorder that causes multiple joint contractures at birth).

Physical Malformations. • Small head (microcephaly). • A prominent back portion of the head (occiput). • Low-set, malformed ears. • Abnormally small jaw (micrognathia). • Cleft lip/cleft palate, upturned nose.

• Narrow eyelid folds (palpebral fissures), widely spaced eyes (ocular hypertelorism), drooping of the upper eyelids (ptosis). • A short breast bone. • Clenched hands • Underdeveloped thumbs and or nails. • In males, undescended testicles.

Genetics • Chromosomal abnormality characterized by the presence of an extra copy of genetic material on the 18th chromosome. • Either in whole (trisomy 18). • Or in part (such as due to translocations).

Karyotype of a female with trisomy 18.

A healthy egg and/or sperm cell contains individual chromosomes, each of which contributes to the 23 pairs of chromosomes needed to form a normal cell with a typical humankaryotype of 46 chromosomes. Numerical errors can arise at either of the two meiotic divisions and cause the failure of a chromosome to segregate into the daughter cells (nondisjunction). This results in an extra chromosome, making the haploid number 24 rather than 23. Fertilization of eggs or insemination by sperm that contain an extra chromosome results in trisomy, or three copies of a chromosome rather than two.

A small percentage of cases occur when only some of the body's cells have an extra copy of chromosome 18, resulting in a mixed population of cells with a differing number of chromosomes. Such cases are sometimes called mosaic Edwards syndrome. Very rarely, a

piece of chromosome 18 becomes attached to another chromosome (translocated) before or after conception. Affected individuals have two copies of chromosome 18 plus extra material from chromosome 18 attached to another chromosome. With a translocation, a person has a partial trisomy for chromosome 18, and the abnormalities are often less severe than for the typical Edwards syndrome.

Prognosis • It is impossible to predict an exact prognosis during pregnancy or the neonatal period. • Half of infants with this condition do not survive beyond the first week of life. • The median lifespan is five to 15 days. • About 8% of infants survive longer than 1 year. • One percent of children live to age 10, typically in less severe cases of the mosaic Edwards syndrome.

DiGeorge SYNDROME

1.Deletion syndrome (DiGeorge Syndrome, Velo- cardio-facial Syndrome) is a disorder caused by the deletion of a small piece of chromosome 22. 2.The deletion occurs near the middle of the chromosome at a location designated q11.2.

Symptoms Common signs and symptoms include : 1. Heart abnormalities that are often present from birth, 2. An opening in the roof of the mouth(a cleft palate) 3. Distinctive facial features. 4. Often experience recurrent infections caused by problems with the immune system 5. Develop autoimmune disorders such as rheumatoid arthritis and Graves disease in which the immune system attacks the body's own tissues and organs 6. They may also have breathing problems 7. Kidney abnormalities 8. Low levels of calcium in the blood 9. A decrease in platelets 10. Significant feeding difficulties 11. Gastrointestinal problems 12. Hearing loss 13. Skeletal differences 14. Mild short stature 15. Abnormalities of spinal bones are also possible.

Palate Skeletal Differences

Causes

1.DiGeorge syndrome or deletion of chromosome 22 is caused by defective genes by birth. 2.Every normal child will have two copies of 22nd chromosome which contains on average 500-800 genes. 3.But a child with this syndrome will have only 30-40 genes on the chromosome 22. 4.Some children will have short deletion of the genes and many will have

very less count of genes on this chromosome. 5.This deletion of genes can occur either from the father or from the mother and this is reflected in the early stages of fetal development. 6.Hence there will be deletion of genes in all the cells formed by this chromosome 22.

Treatment 1. Since it is a genetic disorder, it cannot be cured by any medicine. 2.Symptoms can only be managed by medications, therapy and surgery. 3.A team of health care specialists are needed for correcting/repairing and monitoring the child's development. 4. Heart problems like Ventricular Septal defect can be corrected through surgery 5. Parathyroid problems can be controlled by giving calcium and vitamin D supplements. 6. There can be frequent infections for the child which has to be treated by antibiotics since immune function would be poor during early childhood and there can be cold infection and ear infection for the child. 8. Cleft palate can be corrected by dental surgery. 9. The child should be given therapy for speech, walking and learning. 10. He should attend behavior therapy classes for developing social skills gradually. 11. Psychotherapy and medicines are to be given for 12. Coping and support is absolutely necessary for upbringing such children

Prevention 1. Genetic counseling can be done for the parents who have defective chromosomes to avoid getting pregnant.

PRADER WILLI SYNDROME

• Seven genes (or some subset thereof) on chromosome 15 (q 11–13)are deleted or unexpressed (chromosome 15q partial deletion) on the paternal chromosome

• It was first described in 1956 by Andrea Prader (1919–2001), Heinrich Willi (1900– 1971), Alexis Labhart (1916–1994)), Andrew Ziegler, and Guido Fanconi of Switzerland.

SIGNS & SYMPTOMS Uterus and birth • Reduced fetal movement • Frequent abnormal fetal position • excessive amniotic fluid • caesarean births • Lethargy • Hypotonia • Feeding difficulties • Difficulties establishing respiration Childhood • Delayed milestones/intellectual delay • Excessive sleeping • crossed eyes • Speech delay • Poor physical coordination • Hyperphagia • Excessive weight gain • Sleep disorders • Delayed puberty • Short stature Adulthood • Infertility • Hypogonadism • Sparse pubic hair • Obesity • Hypotonia • Learning disabilities • Prone to diabetes mellitus • Extreme flexibility

• Strong in visual organization and perception, including reading and vocabulary, but spoken language is generally poorer. • A marked skill in completing jigsaw puzzles • Auditory information processing and sequential processing are relatively poor • Psychiatric symptoms-hallucinations, paranoia and depression

PHYSICAL APPEARANCE • Prominent nasal bridge • Small hands and feet • Excess fat • High, narrow forehead • Thin upper lip • Light skin and hair relative to other family members • Lack of complete sexual development • Delayed motor development

DIAGNOSIS • PWS affects approximately 1 in 10,000 to 1 in 25,000 newborns. • Currently, the syndrome is diagnosed through genetic testing • Methylation-specific testing -too young • Misdiagnosed as a variety of other syndromes

TREATMENT • No cure • Infancy- therapies to improve muscle tone. Speech and occupational therapy • The school years a highly structured learning environment • Daily recombinant growth hormone • Obstructive sleep apnea -surgical procedures.

SEX CHROMOSOME DISORDERS

Sex chromosome disorders belong to a group of genetic conditions that are caused or affected by the loss or damage of sex chromosomes (gonosomes).

In humans this may refer to:

- 47,XXX
- 48, XXXX
- 49 XXXXY syndrome
- 49, XXXXX
- Klinefelter's syndrome, XXY
- Turner syndrome, X
- XX gonadal dysgenesis
- XX male syndrome
- XXYY syndrome

- XYY syndrome

Triple X syndrome (also known as **triplo-X**, **trisomy X**, **XXX syndrome**, **47,XXX aneuploidy**) is a form of chromosomal variation characterized by the presence of an extra X chromosome in each cell of a human female. A mosaic form also occurs where only a percentage of the body cells contain XXX while the remainder carry XX. The extent to which an individual is affected by the condition will depend upon the proportion of XXX to XX throughout. Triple X results during division of a parent's reproductive cells and occurs about once in every 1,000 female births. Unlike most other chromosomal conditions (such as Down syndrome), there is usually no distinguishable difference to the naked eye between those with triple X and the rest of the female population.

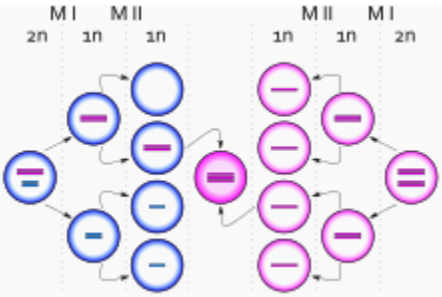
Signs and symptoms

Because the vast majority of Triple X females are never diagnosed, it may be very difficult to make generalizations about the effects of this syndrome. The samples that were studied were small and may be nonrepresentative or biased.

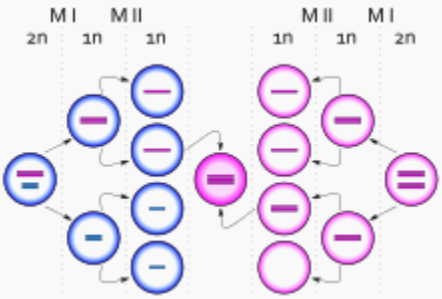
Because of the lyonization, inactivation, and formation of Barr bodies in all female cells, only one X chromosome is active at any time. Thus, Triple X syndrome most often has only mild effects, or has no unusual effects at all. Symptoms may include tall stature; small head (microcephaly); vertical skinfolds that may cover the inner corners of the eyes (epicanthal folds); speech and language learning disabilities, such as dyslexia; or weak muscle tone.[2] The symptoms vary from person to person, with some women being more affected than others. There are seldom any observable physical anomalies in Triple X females, other than being taller than average.

Females with Triple X syndrome are at increased risk of delayed language development, EEG abnormalities, motor coordination problems and auditory processing disorders, andscoliosis. They tend to show accelerated growth until puberty. Premature ovarian failure seems to be more prevalent in these women, but most Triple X females seem to have normal fertility. They are more likely to struggle with personality and psychological problems, and low self-esteem, but these respond well to treatment. Triple X females are at increased risk of poor academic results at school, and some may need special education. Sometimes, they may suffer from anxiety and be very shy, and this may affect their relations with school peers. They seem to feel much better after leaving school. They benefit very much from a stable home environment.

Cause



Problems in male meiosis resulting in a male cell with 2 X-chromosomes.



Problems in female meiosis resulting in a female cell with 3 X-chromosomes.

Triple X syndrome is not inherited, but usually occurs as an event during the formation of reproductive cells (ovum and sperm). An error in cell division called nondisjunction can result in reproductive cells with additional chromosomes. For example, an oocyte or sperm cell may gain an extra copy of the X chromosome as a result of the non-disjunction. If one of these cells contributes to the genetic makeup of a child, the child will have an extra X chromosome in each of her cells. In some cases, trisomy X occurs during cell division in earlyembryonic development.

Some females with triple X syndrome have an extra X chromosome in only some of their cells. These cases are called 46,XX/47,XXXmosaics.

Diagnosis

The vast majority of Triple X women are never diagnosed, unless they undergo tests for other medical reasons later in life. Triple X can be diagnosed by a blood test which is able to look at a person's chromosomes (karyotype).

Triple X syndrome can be diagnosed prenatally through amniocentesis or chorionic villus sampling. In Denmark, between 1970 and 1984, 76% of the prenatally diagnosed fetuses with triple-X were aborted. Between 1985-1987, this figure dropped to 56%. With improved information, the number of abortions diminished. In the Netherlands, between 1991 and 2000, 33% (18/54) of the couples that were confronted with a prenatal diagnosis of 47,XXX elected to

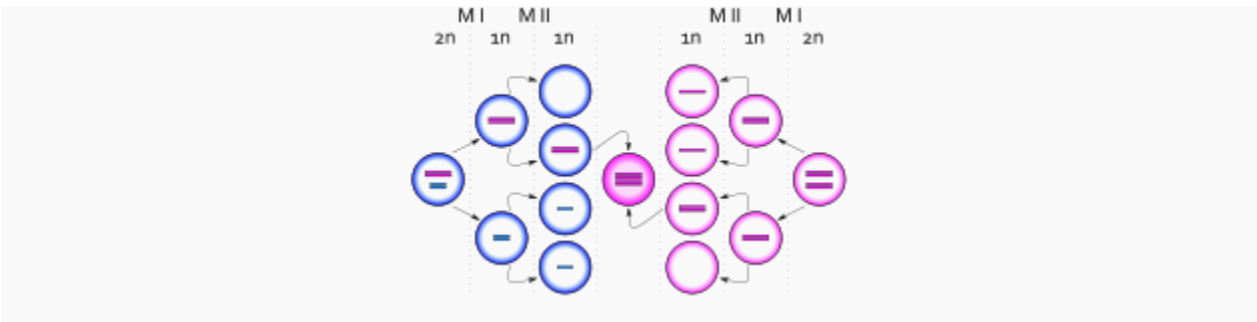
abort. If balanced information is provided to prospective parents, pre-natally, the incidence of voluntary termination (abortion) is reduced.

Epidemiology

Triple X syndrome occurs in around 1 in 1,000 girls. On average, five to ten girls with triple X syndrome are born in the United States each day.

XXXX syndrome (also called **tetrasomy X**, **quadruple X**, or **48, XXXX**) is a rare chromosomal disorder caused by the presence of four X chromosomes instead of two X chromosomes. This condition occurs only in females, as there are no Y chromosomes present. Tetrasomy X was first described in 1961, and since then approximately 100 cases have been reported worldwide. Approximately 60 females have been described in medical literature with this condition.

Causes and diagnosis



Tetrasomy X is a chromosomal aneuploidy, meaning it arises from a defect in meiosis. This can occur when homologous X chromosomes fail to separate in the formation of the egg or sperm. Tetrasomy X is usually suspected based on symptoms present in the individual and is confirmed via karyotyping, which reveals the extra X chromosomes.

Symptoms

Symptoms of tetrasomy X are highly variable, ranging from relatively mild to severe. Symptoms are often similar to those of trisomy X. Physically, tetrasomy X patients tend to have distinctive facial features such as epicanthal folds, flat nasal bridges, upslanting palpebral fissures, midface hypoplasia, small mouths, cleft or high arched palates, delayed or absent teeth, or enamel defects. The majority have also been reported as being longer and taller. Many also show joint and muscle tone abnormalities, including hypotonia and joint looseness in the hips. Skeletal problems may also be present, including abnormal curvatures of the spine. An informal study conducted by Tetrasomy & Pentasomy X Syndrome Information and Support found that 10% of girls had joint laxity in the hips and 20% had joint limitations in a sample size of 20 tetrasomy and pentasomy patients.

Developmentally, tetrasomy X patients frequently show mild delays in the areas of speech development and articulation, language expression and understanding, and reading skills. Delays in motor development are also present, with walking ages ranging from 16 months to 4.5 years. About 50% of patients undergo puberty normally, whereas the other 50% experiences no puberty, partial puberty without secondary sexual characteristics, or complete puberty with menstrual irregularities and/or early menopause (possibly as early as the teens). Medical literature reports four tetra-X pregnancies, two healthy, one with trisomy 21, one stillborn with omphalocele.

In terms of internal organ systems, tetrasomy X patients may have abnormal vision, hearing, circulatory systems, kidneys, or nervous systems. Disorders of the eye includemyopia, nystagmus, coloboma, microphthalmus, or optic nerve hypoplasia. In terms of hearing, patients are more prone to ear infections, sound blockage, or nerve abnormalities. Several cardiac defects have also been reported, including ventricular/atrial septal defects, atresia, hypoplastic right heart syndrome, patent ductus arteriosus, and conotruncal or valvular cardiac defects. Tetrasomy X patients also appear to be more prone to seizure activity, although there is no documented abnormalities in brain function or structure when analyzed using an EEG or MRI.

Treatment and prognosis

The general prognosis for girls with tetrasomy X is relatively good. Due to the variability of symptoms, some tetrasomy X girls are able to function normally, whereas others will need medical attention throughout their lives. Traditionally, treatment for tetrasomy X has been management of the symptoms and support for learning. Most girls are placed on estrogen treatment to induce breast development, arrest longitudinal growth, and stimulate bone formation to prevent osteoporosis. Speech, occupational, and physical therapy may also be needed depending on the severity of the symptoms.

49, XXXXY syndrome is an extremely rare aneuploidic sex chromosomal abnormality. It occurs in approximately 1 out of 85,000 to 100,000 males.

Pathophysiology

As its name indicates, a person with the syndrome has one Y chromosome and four X chromosomes on the 23rd pair, thus having 49 chromosomes rather than the normal 46. As is common with aneuploidy disorders, 49, XXXXY syndrome is often accompanied by intellectual disability. It can be considered a form of Klinefelter syndrome, or a variant of it.

It is genetic, but not hereditary. This means that while the genes of the parents cause the syndrome, there is a small chance of more than one child having the syndrome. The probability of inheriting the disease is about 1%.

The individuals with this syndrome are males, but 49, XXXXX also exists with similar characteristics as the female version.

Effects

Aneuploidy is often fatal, but in this case there is "X-inactivation" where the effect of the additional gene dosage due to the presence of extra X chromosomes is greatly reduced.

Much like Down syndrome the mental effects of 49, XXXXY syndrome vary. Impaired speech and behavioral problems are typical.[5] Those with 49, XXXXY syndrome tend to exhibit infantile secondary sex characteristics with sterility in adulthood and have some skeletal anomalies. Skeletal anomalies include:

- Genu valgum
- Pes cavus
- Fifth finger clinodactyly

The effects also include:

- Cleft palate
- Club feet
- Respiratory conditions
- Short or/and broad neck
- Low birth weight
- Hyperextensible joints
- Short stature
- Narrow shoulders
- Coarse features in older age
- Hypertelorism
- Epicanthal folds
- Prognathism
- Gynecomastia (rare)
- Muscular hypotonia
- Hypoplastic genitalia
- Cryptorchidism
- Congenital heart defects

- A very round face in infancy

XXXXX syndrome (also called **pentasomy X** or **49,XXXXX**) is a type of aneuploidy (an abnormal number of chromosomes) which results in the presence of three additional X chromosomes. The condition was first described in 1963. It is characterized by severe intellectual disability, short stature and abnormalities to the head and face.[1] As these features can be seen in other conditions, karyotyping must be carried out to confirm diagnosis. There have been cases of XXXXX syndrome being misdiagnosed as Down syndrome.

It is an extremely rare condition with no more than 30 patients reported in medical literature.[3] The exact incidence is not known but it may be similar to the rate of 1 in 85,000 seen in males with 49, XXXXY syndrome.

Signs and symptoms

The main characteristics of XXXXX syndrome are intellectual disability, short stature and craniofacial abnormalities. Other physical traits include the following:

- Small head
- Ear abnormalities
- Widely spaced eyes with upward slanting palpebral fissures and epicanthal folds
- Short neck
- Broad nose with a depressed nasal bridge
- Hyperextension of the elbows
- Dental abnormalities and cleft palate[1][5]
- Clinodactyly of the 5th finge
- Deformities of the feet
- Heart defects

Causes

The aneuploidy is thought to be caused by problems occurring during meiosis, either in the mother or in both the mother and father. Successive nondisjunctions have been observed in the mother of at least one patient.[1][3] The features of the syndrome likely arise due to failure of x inactivation and the presence of multiple X chromosomes from the same parent causing problems with parental imprinting. In theory, X inactivation should occur and leave only one X chromosome active in each cell. However, failure of this process has been observed in one individual studied. The reason for this is thought to be the presence of an unusually large, and imbalanced, number of X chromosomes interfering with the process.[3]

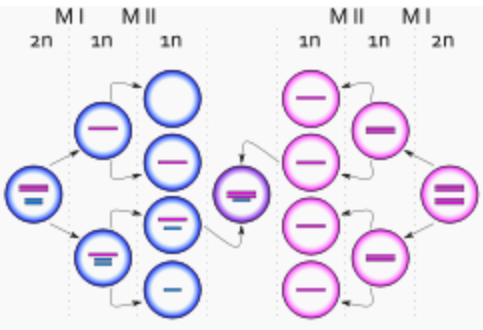
Klinefelter syndrome or **Klinefelter's syndrome** (**KS**) (/ˈklaɪnfɛltər/) also known as **47,XXY** or **XXY**, is the set of symptoms that result from two or more X chromosomes in males. The primary feature is sterility.[1] Often symptoms may be subtle and many people do not realize they are affected. Sometimes symptoms are more prominent and may include weaker muscles, greater height, poor coordination, less body hair, smaller genitals, breast growth, and less interest in sex. Often it is only at puberty that these symptoms are noticed.Intelligence is usually normal; however, reading difficulties and problems with speech are more common. Symptoms are typically more severe if three or more X chromosomes are present.

Klinefelter syndrome usually occurs randomly. An older mother might increase the risk slightly. The condition is not inherited from one's parents.[4] The underlying mechanisms involves at least one extra X chromosome in addition to a Y chromosome such that there is a total of 47 or more chromosomes rather than usual 46. KS is diagnosed by the genetic test known as a karyotype.

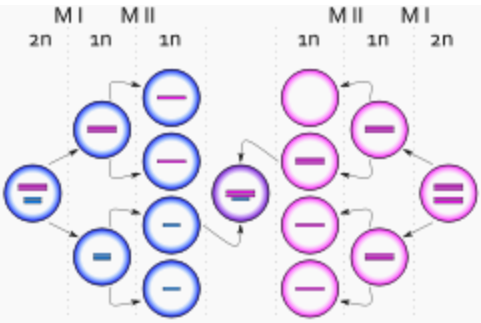
While there is no cure, a number of treatments may help. Physical therapy, speech and language therapy, counselling, and adjustments of teaching methods may be useful. Testosterone replacement may be used in those who have significantly low levels. Enlarged breasts may be removed by surgery. About half of males affected with the help of assisted reproductive technology have a chance of having children; however, this is expensive and carries risks.[7] The condition has a nearly normal life expectancy.

Klinefelter syndrome is one of the most common chromosomal disorders, occurring in 1:500 to 1:1000 live male births.[4][8] It is named after Harry Klinefelter who identified the condition in the 1940s.[9] 1956 saw the identification of the extra X chromosome.[10]Mice also can have the XXY syndrome, making them a useful research model.[11]

Cause



Birth of a cell with karyotype XXY due to a non-disjunction event of one X chromosome from a Y chromosome duringmeiosis I in the male.



Birth of a cell with karyotype XXY due to a non-disjunction event of one X chromosome during meiosis II in the female.

The extra chromosome is retained because of a nondisjunction event during paternal or maternal meiosis I (gametogenesis). Nondisjunction occurs when homologous chromosomes, in this case the X and Y or two X sex chromosomes, fail to separate, producing a sperm with an X and a Y chromosome or an egg with two X chromosomes. Fertilizing a normal (X) egg with this sperm produces an XXY offspring (Klinefelter). Fertilizing a double X egg with a normal sperm also produces an XXY offspring (Klinefelter).

Another mechanism for retaining the extra chromosome is through a nondisjunction event during meiosis II in the egg. Nondisjunction will occur when sister chromatids on the sex chromosome, in this case an X and an X, fail to separate. (meiosis) An XX egg is produced which, when fertilized with a Y sperm, yields XXY offspring. This XXY chromosome arrangement is one of the most common genetic variations from the XY karyotype, occurring in about 1 in 500 live male births.[12] See also Triple X syndrome.

Diagnosis

About 10% of Klinefelter cases are found by prenatal diagnosis. The first clinical features may appear in early childhood or, more frequently, during puberty, such as lack of secondary sexual characters and aspermatogenesis,[31] while tall stature as a symptom can be hard to diagnose during puberty. Despite the presence of small testes, only a quarter of the affected males are recognized as having Klinefelter syndrome at puberty and 25% received their diagnosis in late adulthood: about 64% affected individuals are not recognized as such. Often the diagnosis is made accidentally as a result of examinations and medical visits for reasons not linked to the condition.

The standard diagnostic method is the analysis of the chromosomes' karyotype on lymphocytes. In the past, the observation of the Barr body was common practice as well. To confirm mosaicism, it is also possible to analyze the karyotype usingdermal fibroblasts or testicular tissue.

Other methods may be: research of high serum levels of gonadotropins (follicle-stimulating hormone and luteinizing hormone), presence of azoospermia, determination of the sex chromatin, and prenatally via chorionic villus sampling oramniocentesis. A 2002 literature review of elective abortion rates found that approximately 58% of pregnancies in the United States with a diagnosis of Klinefelter syndrome were terminated.

Treatment

The genetic variation is irreversible, however, individuals who want to look more masculine can take testosterone. Treating adolescents with implants of controlled release testosterone has shown good results when appropriately monitored. Hormone therapy is also useful in preventing the onset of osteoporosis.

Often individuals that have noticeable breast tissue or hypogonadism experience depression and/or social anxiety because they are outside of social norms. An academic term for this is psychosocial morbidity. At least one study indicates that planned and timed support should be provided for young men with Klinefelter syndrome to ameliorate current poor psychosocial outcomes. The surgical removal of the breasts may be considered for both the psychological reasons and to reduce the risk of breast cancer.

The use of behavioral therapy can mitigate any language disorders, difficulties at school and socialization. An approach by occupational therapy is useful in children with Down syndrome who have dyspraxia motor.[44]

Prognosis

Children with XXY differ little from other children. Although they can face problems during adolescence, often emotional and behavioral, and difficulties at school, most of them can achieve full independence from their families in adulthood. Most can lead a normal, healthy life.

The results of a study carried out on 87 Australian adults with the syndrome shows that those who have had a diagnosis and appropriate treatment from a very young age had a significant benefit with respect to those who had been diagnosed in adulthood.[47]

Epidemiology

This syndrome, evenly spread in all ethnic groups, has a prevalence of 1-2 subjects every 1000 males in the general population. 3.1% of infertile males have Klinefelter syndrome. The syndrome is also the main cause of male hypogonadism.

According to a meta-analysis, the prevalence of the syndrome has increased over the past decades; however, this does not appear to be correlated with the increase of the age of the mother

at conception, as no increase was observed in the prevalence of other trisomies of sex chromosomes (XXX and XYY).

Turner syndrome (**TS**) also known as **Ullrich–Turner syndrome**, **gonadal dysgenesis**, and **45,X**, is a condition in which a femaleis partly or completely missing an X chromosome. Signs and symptoms vary among those affected. Often, a short and webbed neck, low-set ears, low hairline at the back of the neck, short stature, and swollen hands and feet are seen at birth. Typically they arewithout menstrual periods, do not develop breasts, and are unable to have children. Heart defects, diabetes, and low thyroid hormone occur more frequently. Most people with TS have normal intelligence. Many, however, have troubles with spatial visualization such as that needed for mathematics. Vision and hearing problems occur more often.

Turner syndrome is not usually inherited from a person's parents.[4] No environmental risks are known and the mother's age does not play a role. Turner syndrome is due to a chromosomal abnormality in which all or part of one of the X chromosomes is missing or altered. While most people have 46 chromosomes, people with TS usually have 45. The chromosomal abnormality may be present in just some cells in which case it is known as TS with mosaicism. In these cases, the symptoms are usually fewer and possibly none occur at all. Diagnosis is based on physical signs and genetic testing.

No cure for Turner syndrome is known. Treatment, however, may help with symptoms. Human growth hormone injections during childhood may increase adult height. Estrogen replacement therapy can promote development of the breasts and hips. Medical care is often required to manage other health problems with which TS is associated.

Turner syndrome occurs in between one in 2000 and one in 5000 females at birth. All regions of the world and cultures are affected about equally. People with TS have a shorter life expectancy, mostly due to heart problems and diabetes.[3] Henry Turnerfirst described the condition in 1938. In 1964, it was determined to be due to a chromosomal abnormality.

f the following common symptoms of Turner syndrome, an individual may have any combination of symptoms and is unlikely to have all symptoms.

- Short stature
- Lymphedema (swelling) of the hands and feet of a newborn
- Broad chest (shield chest) and widely spaced nipples
- Low hairline
- Low-set ears
- Reproductive sterility

- Rudimentary ovaries gonadal streak (underdeveloped gonadal structures that later become fibrotic)
- Amenorrhoea, the absence of a menstrual period
- Increased weight, obesity
- Shortened metacarpal IV
- Small fingernails
- Characteristic facial features
- Webbed neck from cystic hygroma in infancy
- Aortic valve stenosis
- Coarctation of the aorta
- Bicuspid aortic valve
- Horseshoe kidney
- Visual impairments - sclera, cornea, glaucoma, etc.
- Ear infections and hearing loss
- High waist-to-hip ratio (the hips are not much bigger than the waist)
- Attention deficit hyperactivity disorder (problems with concentration, memory, attention with hyperactivity seen mostly in childhood and adolescence)
- Nonverbal learning disability (problems with math, social skills, and spatial relations)

Other features may include a small lower jaw (micrognathia), cubitus valgus, soft upturned nails, palmar crease, and drooping eyelids. Less common are pigmented moles, hearing loss, and a high-arch palate (narrow maxilla). Turner syndrome manifests itself differently in each female affected by the condition; therefore, no two individuals share the same features.

While most of the physical findings are harmless, significant medical problems can be associated with the syndrome.

Prenatal

Despite the excellent postnatal prognosis, 99% of Turner-syndrome conceptions are thought to end in spontaneous abortion or stillbirth, and as many as 15% of all spontaneous abortions have the 45,X karyotype. Among cases that are detected by routine amniocentesis or chorionic villus sampling, one study found that the prevalence of Turner syndrome among tested pregnancies was 5.58 and 13.3 times higher, respectively, than among live neonates in a similar population.[16]

Cause[edit]

Turner syndrome is caused by the absence of one complete or partial copy of the X chromosome in some or all the cells. The abnormal cells may have only one X (monosomy) (45,X) or they may be affected by one of several types of partial monosomy like a deletion of the short p arm of

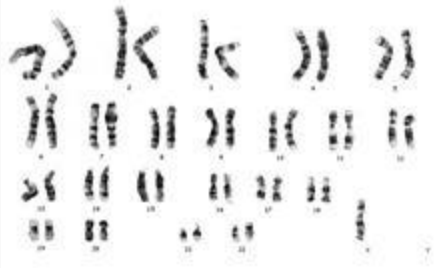
one X chromosome (46,X,del(Xp)) or the presence of anisochromosome with two q arms (46,X,i(Xq)). In mosaic individuals, cells with X monosomy (45,X) may occur along with cells that are normal (46,XX), cells that have partial monosomies, or cells that have a Y chromosome (46,XY). The presence of mosaicism is estimated to be relatively common in affected individuals (67–90%).

Inheritance

In the majority of cases where monosomy occurs, the X chromosome comes from the mother. This may be due to a nondisjunction in the father. Meiotic errors that lead to the production of X with p arm deletions or abnormal Y chromosomes are also mostly found in the father. Isochromosome X or ring chromosome X on the other hand are formed equally often by both parents. Overall, the functional X chromosome mostly comes from the mother.

Diagnosis

Prenatal



45,X karyotype, showing an unpaired X at the lower right

Turner syndrome may be diagnosed by amniocentesis or chorionic villus sampling during pregnancy.

Usually, fetuses with Turner syndrome can be identified by abnormal ultrasound findings (*i.e.*, heart defect, kidney abnormality, cystic hygroma, ascites).

Although the recurrence risk is not increased, genetic counseling is often recommended for families who have had a pregnancy or child with Turner syndrome.

Postnatal

Turner syndrome can be diagnosed postnatally at any age. Often, it is diagnosed at birth due to heart problems, an unusually wide neck or swelling of the hands and feet. However, it is also common for it to go undiagnosed for several years, typically until the girl reaches the age of puberty/adolescence and she fails to develop properly (the changes associated with puberty do not occur). In childhood, a short stature can be indicative of Turner syndrome.[43]

A test, called a karyotype or a chromosome analysis, analyzes the chromosomal composition of the individual. This is the test of choice to diagnose Turner syndrome.

Treatment

As a chromosomal condition, there is no cure for Turner syndrome. However, much can be done to minimize the symptoms. For example:

- Growth hormone, either alone or with a low dose of androgen, will increase growth and probably final adult height. Growth hormone is approved by the U.S. Food and Drug Administration for treatment of Turner syndrome and is covered by many insurance plans.[44][45] There is evidence that this is effective, even in toddlers.

- Estrogen replacement therapy such as the birth control pill, has been used since the condition was described in 1938 to promote development of secondary sexual characteristics. Estrogens are crucial for maintaining good bone integrity, cardiovascular health and tissue health. Women with Turner Syndrome who do not have spontaneous puberty and who are not treated with estrogen are at high risk for osteoporosis and heart conditions.

- Modern reproductive technologies have also been used to help women with Turner syndrome become pregnant if they desire. For example, a donor egg can be used to create an embryo, which is carried by the Turner syndrome woman.
- Uterine maturity is positively associated with years of estrogen use, history of spontaneous menarche, and negatively associated with the lack of current hormone replacement therapy.

Epidemiology

Approximately 99 percent of all fetuses with Turner syndrome result in spontaneous termination during the first trimester. Turner syndrome accounts for about 10 percent of the total number of spontaneous abortions in the United States. The incidence of Turner syndrome in live female births is believed to be around 1 in 2000.

History

The syndrome is named after Henry Turner, an endocrinologist from Illinois, who described it in 1938. In Europe, it is often called **Ullrich–Turner syndrome** or even **Bonnevie–Ullrich–Turner syndrome** to acknowledge that earlier cases had also been described by European doctors.

The first published report of a female with a 45,X karyotype was in 1959 by Dr. Charles Ford and colleagues in Harwell, Oxfordshire and Guy's Hospital in London.It was found in a 14-year-old girl with signs of Turner syndrome.

XX gonadal dysgenesis is a type of female hypogonadism in which no functional ovaries are present to induce puberty in an otherwise normal girl whose karyotype is found to be 46,XX.

With nonfunctional *streak* ovaries she is low in estrogen levels (hypoestrogenic) and has high levels of FSH and LH. Estrogen and progesterone therapy is usually then commenced..

This syndrome is inherited as an autosomal disease. It affects both males and females but the phenotype differs. In both sexes sensorineural deafness occurs but in females ovarian dysgenesis also occurs.

XX GD as a form of "pure gonadal dysgenesis"

The term "pure gonadal dysgenesis" (PGD) has been used to distinguish a group of patients from gonadal dysgenesis related to Turner syndrome. In the latter a distinct chromosomal aberration is present, while in PGD the chromosomal constellation is either 46,XX or 46,XY. Thus XX gonadal dysgenesis is also referred to as *PGD, 46 XX*, and XY gonadal dysgenesis as *PGD, 46,XY* or Swyer syndrome. Patients with PGD have a normal chromosomal constellation but may have localized genetic alterations.

Pathogenesis

The cause of the condition is often unclear. There are cases where abnormalities in the FSH-receptor have been reported. Apparently either the germ cells do not form or interact with the gonadal ridge or undergo accelerated atresia so that at the end of childhood only a streak gonad is present, unable to induce pubertal changes. As girls' ovaries produce no important body changes before puberty, there is usually no suspicion of a defect of the reproductive system until puberty fails to occur.

Familial cases of XX gonadal dysgenesis are on record.

In one family mutations in the mitochondrial histidyl tRNA synthetase have been described as the cause.

Perrault syndrome

In 1951, Perrault reported the association of gonadal dysgenesis and deafness, now called **Perrault syndrome**.

Diagnosis

Because of the inability of the streak gonads to produce sex hormones (both estrogens and androgens), most of the secondary sex characteristics do not develop. This is especially true of estrogenic changes such as breast development, widening of the pelvis and hips, and menstrual periods. Because the adrenal glands can make limited amounts of androgens and are not affected by this syndrome, most of these girls will develop pubic hair, though it often remains sparse.

Evaluation of delayed puberty usually reveals the presence of pubic hair, but elevation of gonadotropins, indicating that the pituitary is providing the signal for puberty but the gonads are failing to respond. The next steps of the evaluation usually include checking a karyotype and imaging of the pelvis. The karyotype reveals XX chromosomes and the imaging demonstrates the presence of a uterus but no ovaries (the streak gonads are not usually seen by most imaging). At this point it is usually possible for a physician to make a diagnosis of XX gonadal dysgenesis.

Treatment

The consequences to the girl with XX gonadal dysgenesis:

1. Her gonads cannot make estrogen, so her breasts will not develop and her uterus will not grow and menstruate until she is given estrogen. This is often given through the skin now.
2. Her gonads cannot make progesterone, so her menstrual periods will not be predictable until she is given a progestin, still usually as a pill.
3. Her gonads cannot produce eggs so she will not be able to conceive children naturally. A woman with a uterus but no ovaries may be able to become pregnant by implantation of another woman's fertilized egg (embryo transfer).

Related conditions

XX gonadal dysgenesis is related to the Swyer syndrome inasmuch as both conditions have the same phenotype and clinical issues; however in Swyer syndrome the karyotype is 46,XY, and thus gonadectomy is recommended.

In Turner syndrome there is a demonstrable abnormality in or absence of one of the sex chromosomes that is the cause of the development of gonadal dysgenesis. In contrast XX gonadal dysgenesis has a normal female chromosome situation.

Another type of XX gonadal dysgenesis is known as 46,XX gonadal dysgenesis epibulbar dermoid, which follows the similar symptoms as the regular syndrome, though it also shows signs of epibulbar dermoid (eye disorder).[6][7][8] It has been suggested to be a new type of syndrome.

XX male syndrome (also called **de la Chapelle syndrome**, for Albert de la Chapelle, who characterized it in 1972) is a rare sex chromosomal disorder. Usually, it is caused by unequal crossing over between X and Y chromosomes during meiosis in the father, which results in the X chromosome containing the normally-male SRY gene. When this X combines with a normal X from the mother during fertilization, the result is an XX male.

This syndrome occurs in approximately four or five in 100,000 individuals, making it less common than Klinefelter syndrome.

Presentation

Symptoms usually include small testes and subjects are invariably sterile. Individuals with this condition sometimes have feminine characteristics, with varying degrees ofgynecomastia but with no intra-abdominal Müllerian tissue. According to research at the University of Oklahoma health science centers, most XX males are not stereotypically feminine and are typical boys and men, though other reports suggest that facial hair growth is usually poor and libido is diminished, with notable exceptions.

Clinical diagnosis

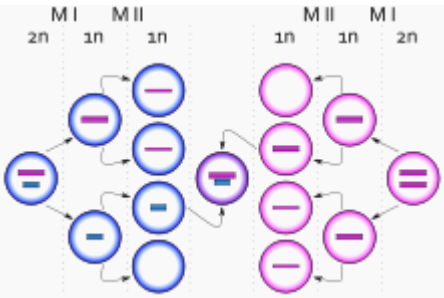
- Standard XX karyotype in two tissues (with at least one, or both, containing the male SRY gene)
- Male external genitalia, sometimes showing hypospadias

- Two testes which may or may not have descended the inguinal canal. Most XX males have descended testes.
- Absence of Müllerian tissue

Pathophysiology

Males typically have one X chromosome and one Y chromosome in each diploid cell of their bodies. Females typically have two X chromosomes. XX males have two X chromosomes, with one of them containing genetic material from the Y chromosome, making them phenotypically male; they are genetically female but otherwise appear to be male.

48, XXYY syndrome is a sex chromosome anomaly in which males have an extra X and Y chromosome. Human cells usually contain two sex chromosomes, one from the mother and one from the father. Usually, females have two X chromosomes (XX) and males have one X and one Y chromosome (XY). The appearance of at least one Y chromosome with a properly functioning SRY gene makes a male. Therefore, XXYY normally only affects males. Males affected with XXYY syndrome have 48 chromosomes instead of the typical 46. This is why XXYY syndrome is sometimes written as 48, XXYY syndrome. It is estimated that XXYY affects one in every 18,000–40,000 male births.



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History

The first published report of a boy with a 48, XXYY karyotype was by Sylfest Muldal and Charles H. Ockey in Manchester, England in 1960. It was described in a 15-year-old mentally challenged boy who showed signs of Klinefelter syndrome; however, chromosome testing revealed 48, XXYY instead of the 47, XXY arrangement known to be the cause. Because of this,

48, XXYY syndrome was originally considered a variation of Klinefelter syndrome. Shared physical and medical features resulting from the presence of an extra X chromosome include tall stature, the development of testosterone deficiency in adolescence and/or adulthood (hypergonadotropic hypogonadism), and infertility. However, recent research shows some important differences in males with 48, XXYY compared to 47, XXY. The most important differences result from the effects of the extra X and Y chromosome on neurodevelopment, leading to higher rates of developmental delays in early childhood, learning disability or intellectual disability, adaptive functioning (life skills) difficulties, neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD) or autism spectrum disorders, and psychological/behavioral problems including anxiety, depression, and mood dysregulation. Also, a larger percentage of males with XXYY have additional medical problems such as seizures, congenital elbow malformations (radioulnar synostosis), and tremor compared to males with XXY. XXYY is still considered a variation of Klinefelter syndrome by some definitions, mainly because the pathophysiology of the testicular dysfunction has not been shown to differ from 47, XXY, and the most current research does not suggest that there should be any differences in the evaluation and treatment of testosterone deficiency in 48, XXYY compared to 47, XXY.[4] However, for the psychological and behavioral symptoms of XXYY syndrome, more extensive evaluations, interventions, and supports are usually needed compared to 47, XXY due to more complex neurodevelopmental involvement. There is significant variability between individuals in the number and severity of the medical and neurodevelopmental problems associated with XXYY, and some individuals have mild symptoms while others are more significantly affected.[1]

Presentation

- Developmental delays
- Speech impairment
- Behavior outburst and mood swings
- Learning disabilities
- Intellectual impairment
- ADHD symptoms
- Autism spectrum disorders

- Tall
- Scoliosis
- Clinodactyly
- Low muscle tone
- Flat feet
- Sterility
- Delayed sexual development
- Undescended testes
- Low testosterone

Diagnosis

A karyotype is done to diagnose XXYY syndrome. Treatment consists of medications, behavioral therapies and intensive community support.

XYY syndrome is a genetic condition in which a human male has an extra male (Y) chromosome, giving a total of 47 chromosomes instead of the more usual 46. This produces a **47,XYY** karyotype, which occurs every 1 in 1,000 male births.

Some medical geneticists question whether the term "syndrome" is appropriate for this condition because its clinical phenotype is normal and the vast majority of XYY males do not know their karyotype.

Signs and symptoms

Physical traits

People with the 47,XYY karyotype have an increased growth velocity from early childhood, with an average final height approximately 7 cm (3") above expected final height.[4] InEdinburgh, Scotland, eight 47,XYY boys born 1967–1972 and identified in a newborn screening programme had an average height of 188.1 cm (6'2") at age 18—their fathers' average height was 174.1 cm (5'8½"), their mothers' average height was 162.8 cm (5'4"). The increased gene dosage of three X/Y chromosome pseudoautosomal region(PAR1) SHOX genes

has been postulated as a cause of the increased stature seen in all three sex chromosome trisomies: 47,XXX, 47,XXY, and 47,XYY.

Severe acne was noted in a very few early case reports, but dermatologists specializing in acne now doubt the existence of a relationship with 47,XYY.

Testosterone levels (prenatally) are normal in 47,XYY males. Most 47,XYY males have normal sexual development and usually have normal fertility.

Behavioral characteristics

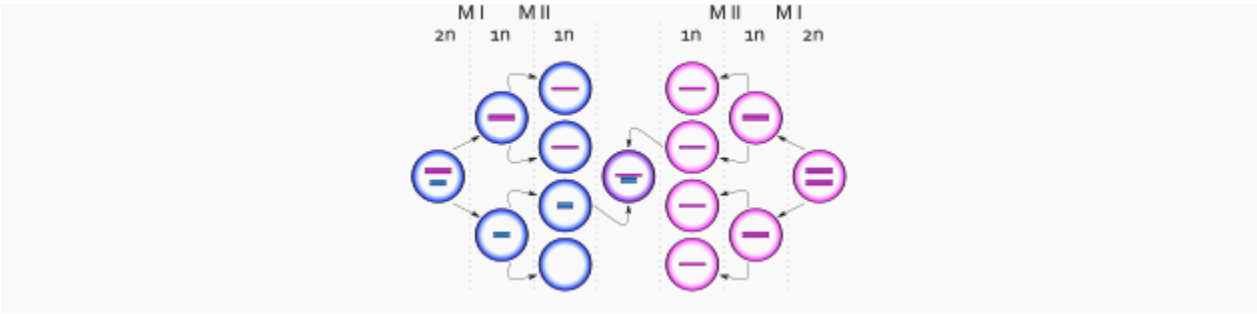
In contrast to the other common sex chromosome aneuploidies—47,XXX, 45,X (Turner syndrome), and 47,XXY (Klinefelter syndrome)—the average IQ scores of 47,XYY boys identified by newborn screening programs were not reduced compared to the general population.. In a summary of six prospective studies of 47,XYY boys identified by newborn screening programmes, twenty-eight 47,XYY boys had an average 100.76 verbal IQ, 108.79 performance IQ, and 105.00 full-scale IQ. In a systematic reviewincluding two prospective studies of 47,XYY boys identified by newborn screening programs and one retrospective study of 47,XYY men identified by screening men over 184 cm (6'½") in height, forty-two 47,XYY boys and men had an average 99.5 verbal IQ and 106.4 performance IQ.

In prospective studies of 47,XYY boys identified by newborn screening programs, the IQ scores of 47,XYY boys were usually slightly lower than their siblings. In Edinburgh, fifteen 47,XYY boys with siblings identified in a newborn screening program had an average 104.0 verbal IQ and 106.7 performance IQ, while their siblings had an average 112.9 verbal IQ and 114.6 performance IQ.

Approximately half of 47,XYY boys identified by newborn screening programs had learning difficulties—a higher proportion than found among siblings and above-average-IQ control groups. In Edinburgh, 54% of 47,XYY boys (7 of 13) identified in a newborn screening program received remedial reading teaching compared to 18% (4 of 22) in an above-average-IQ control group of 46,XY boys matched by their father's social class. In Boston, USA 55% of 47,XYY boys (6 of 11) identified in a newborn screening program had learning difficulties and received part-time resource room help compared to 11% (1 of 9) in an above-average-IQ control group of 46,XY boys with familial balanced autosomal chromosome translocations.

Developmental delays and behavioral problems are also possible, but these characteristics vary widely among affected boys and men, are not unique to 47,XYY and are managed no differently from in 46,XY males. Aggression is not seen more frequently in 47,XYY males.

Cause



47,XYY is not inherited, but usually occurs as a random event during the formation of sperm cells. An incident in chromosome separation during anaphase II (of meiosis II) called nondisjunction can result in sperm cells with an extra copy of the Y-chromosome. If one of these atypical sperm cells contributes to the genetic makeup of a child, the child will have an extra Y-chromosome in each of the body's cells.

In some cases, the addition of an extra Y-chromosome results from nondisjunction during cell division during a post-zygotic mitosis in early embryonic development. This can produce 46,XY/47,XYY mosaics.

Epidemiology

Around 1 in 1,000 boys are born with a 47,XYY karyotype. The incidence of 47,XYY is not affected by advanced paternal or maternal age.

http://www.slideshare.net/ishapunalekar/autosomal-abnormalities

https://en.wikipedia.org/wiki/Sex_chromosome_disorders

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SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY

UNIT – III - Medical Biotechnology – SBB3101

Subject Name: Medical Biotechnology Subject Code: SBB3101

SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY SCHOOL OF BIO & CHEMICAL ENGINEERING

SBB3101	MEDICAL BIOTECHNOLOGY	L	T	P	Credits	Total Marks
		4	0	0	4	100

COURSE OBJECTIVES

- To study about the medicinal approach of Biotechnology and recent advancements in diagnosis.

UNIT 1 ANIMAL CELL CULTURE
12 Hrs.

Animal cell culture-media, maintenance and culture of primary, secondary and continuous cell lines- organ culture-applications- cancer cell lines- apoptosis. Tissue Engineering – Skin, Liver, Pancreas. Assisted reproductive technology- Pregnancy diagnosis.

UNIT 2 CHROMOSOMAL ABNORMALITIES
12 Hrs.

Chromosomal disorders – Gene controlled diseases –Identification of disease genes Haemophilia, DMD, Alzheimer's – Molecular basis of human diseases: Pathogenic mutations – Oncogenes – Loss of function – Tumour Suppressor Genes Immunopathology: Hepatitis, Autoimmune Disorders.

UNIT 3 DIAGNOSTIC TECHNIQUES **12 Hrs.**

Prenatal diagnosis – Invasive techniques and Non-invasive techniques – Diagnosis of pathogenic microbes: Classical and modern methods- Diagnosis using protein and enzyme markers, DNA/RNA based diagnosis – Molecular markers – Microarray technology – genomic and cDNA arrays.

UNIT 4 PREVENTION AND TREATMENT **12 Hrs.**

Vaccines-conventional, recombinant, synthetic peptide, anti-idiotype, DNA vaccines Deletion mutant and vaccinia vector vaccine- Antibiotics-mode of action- antibacterial, antifungal, antiviral, antitumor antibiotics- synthetic chemotherapeutic agent development of microbial resistance to antibiotics.

UNIT 5 MODERN MEDICINE **12 Hrs.**

Hybridoma technique for MCAb production and applications- Gene therapy: Exvivo, In vivo, In situ- Cell and tissue engineering- Stem cell therapy- Nanomedicines- Gene products in medicine – Humulin, Erythropoietin, Growth Hormone/Somatostatin, tPA, Interferon.

Max Hours.60

TEXT / REFERENCE BOOKS:

1. Ramasamy, P. "Trends in Biotechnology", University of Madras, Pearl press, 2002. 2. Trevan. "Biotechnology". Tata McGraw-Hill, 2005.
2. Betty Forbes, Danial SAHM Alics Weinfield, Bailey 2007. Scott's diagnostic microbiology, 12th edition Mosby.
3. Jogdand, S. N. Medical Biotechnology, Himalaya Publishing house, Mumbai, 2005. 2. Click, B. R. and Pasternak. Molecular Biotechnology: Principle and applications of recombinant DNA. ASM Press, 2010.

END SEMESTER EXAMINATION QUESTION PAPER PATTERN

Max. Marks: 100 **Exam Duration: 3 Hrs.**
PART A: 10 questions of 2 marks each - No choice **20 Marks**
PART B: 2 questions from each unit of internal choice; each carrying 16 marks **80 Marks**

Unit 3: Diagnostic Techniques

3.1 Prenatal Diagnosis

01. Approximately 3% to 5% of pregnancies are complicated by birth defects or genetic disorders. Chromosomal abnormalities are present in approximately 1 in 150 live births, and congenital malformations remain the leading cause of infant death and a leading cause of childhood death. These chromosomal abnormalities include aneuploidy (defined as having one or more extra or missing chromosomes), translocations, duplications, and deletions.

02. The most common chromosomal disorder is trisomy 21 (Down syndrome), with an incidence of 1 per 800 live births. Trisomy 13 and 18 can also result in live births, though with a significantly lower incidence. Sex chromosome aneuploidies are less common than autosomal aneuploidies. The only known viable monosomy is monosomy X (Turner syndrome). Incidences are described in Table 1.

Table 1: Incidence of common aneuploidies

Trisomy 21	1 in 800 live births
Trisomy 18	1 in 7500 live births
Trisomy 13	1 in 15,000 live births
Monosomy X (Turner syndrome)	1 in 5000 girls
Trisomy X	1 in 1000 girls
XXY (Klinefelter syndrome)	1 in 1000 boys
XYY	1 in 1000 boys

Data from Nussbaum RL, McInnes RR, Willard HF. Thompson & Thompson genetics in medicine. 7th edition. Philadelphia: Saunders/Elsevier; 2007.

03. Risk of aneuploidy increases with maternal age (Table 2). Other factors also influence patients' risk in any given pregnancy, including the presence of birth defects or soft markers on ultrasound and past obstetric history, particularly if it is notable for a prior pregnancy affected by aneuploidy or another genetic disorder. A past family history of aneuploidy increases current pregnancy risk of aneuploidy, especially if a parent is a balanced robertsonian translocation carrier, though most cases are sporadic and secondary to chromosomal nondisjunction.

Table 2: Risk of aneuploidy by maternal age

Maternal Age at EDD (y)	Risk of Trisomy 21	Risk of Other Chromosomal Abnormality
20	1:1480	1:525
25	1:1340	1:475
30	1:940	1:384
35	1:353	1:178
40	1:85	1:62
45	1:35	1:18

Abbreviation: EDD, estimated date of delivery.

Adapted from Practice bulletin no. 163: screening for fetal aneuploidy. Obstet Gynecol 2016; 127(5): e124.

04. Patients report many different motivations for pursuing aneuploidy screening or prenatal diagnosis. Some may choose pregnancy termination if the defect is identified at an early enough gestational age. Others may choose to pursue screening or testing to allow them time to process the diagnosis and seek experienced clinicians who may be able to aid them in preparation for caring for an affected infant and to care for their child after delivery. Some birth defects, such as some neural tube defects, may be eligible for prenatal treatment with subsequently improved neonatal outcomes.

05. All patients choosing to undergo screening or testing should receive counseling regarding risks, benefits, and limitations of their chosen testing plan from their health care provider or genetic counselor. It is important to note that aneuploidy screening and testing decisions are heavily value driven; a frank discussion of the benefits, risks, and limitations of tests is a key in ensuring that care is appropriate for each patient’s individual goals.

3.2 Molecular Markers of Cancer

01. What are tumor markers?

1. A tumor marker is anything present in or produced by cancer cells or other cells of the body in response to cancer or certain benign (noncancerous) conditions that provides information about a cancer, such as how aggressive it is, whether it can be treated with a targeted therapy, or whether it is responding to treatment.

2. Tumor markers have traditionally been proteins or other substances that are made by both normal and cancer cells but at higher amounts by cancer cells. These can be found in the blood, urine, stool, tumors, or other tissues or bodily fluids of some patients with cancer. Increasingly, however, genomic markers such as tumor gene mutations, patterns of tumor gene expression, and nongenetic changes in tumor DNA, are being used as tumor markers.

02.How are tumor markers used in cancer care?

1. There are 2 main types of tumor markers that have different uses in cancer care:

    1. Circulating tumor markers:

        1. Can be found in the blood, urine, stool, or other bodily fluids of some patients with cancer. Circulating tumor markers are used to:

            1. estimate prognosis
            2. detect cancer that remains after treatment (residual disease) or that has returned after treatment
            3. assess the response to treatment
            4. monitor whether a cancer has become resistant to treatment

2. Although an elevated level of a circulating tumor marker may suggest the presence of cancer, this alone is not enough to diagnose cancer. For example, noncancerous conditions can sometimes cause the levels of certain tumor markers to increase. In addition, not everyone with a particular type of cancer will have a higher level of a tumor marker associated with that cancer. Therefore, measurements of circulating tumor markers are usually combined with the results of other tests, such as biopsies or imaging, to diagnose cancer.

3. Tumor markers may also be measured periodically during cancer therapy. For example, a decrease in the level of a circulating tumor marker may indicate that the cancer is responding to treatment, whereas an increasing or unchanged level may indicate that the cancer is not responding.

4. Examples of commonly used circulating tumor markers include calcitonin (measured in blood), which is used to assess treatment response, screen for recurrence, and estimate prognosis in medullary thyroid cancer; CA-125 (measured in blood), to monitor how well cancer treatments are working and if cancer has come back in ovarian cancer; and beta-2-microglobulin (measured in blood, urine, or cerebrospinal fluid), to estimate prognosis and follow response to treatment for multiple myeloma, chronic lymphocytic leukemia, and some lymphomas.

2. Tumor tissue markers:

    1. Are found in the actual tumors themselves, typically in a sample of the tumor that is removed during a biopsy. Tumor tissue markers are used to:

        1. diagnose, stage, and/or classify cancer
        2. estimate prognosis
        3. select an appropriate treatment (eg, treatment with a targeted therapy)

2. In some types of cancer, the level of a tumor marker reflects the stage (extent) of the disease and/or the patient's prognosis (likely outcome or course of disease). An example is alpha-fetoprotein, which is measured in blood to assess stage, estimate prognosis, and follow response to treatment of germ cell tumors. More information about cancer staging is available on the Staging page.

3. Tumor markers may be measured before treatment to help doctors plan the appropriate therapy. For example, some tests, called companion diagnostics, which have been developed alongside their respective targeted therapy drug, are used to determine whether treatment with a particular targeted therapy is appropriate.

4. Examples of commonly used tumor tissue markers include estrogen receptor and progesterone receptor (breast cancer), used to determine whether treatment with hormone therapy and some targeted therapies is appropriate; EGFR gene mutation analysis (non-small cell lung cancer), to help determine treatment and estimate prognosis; and PD-L1 (many cancer types), to determine whether treatment with a type of targeted therapy called an immune checkpoint inhibitor is appropriate.

03.How are tumor markers measured?

1. A doctor takes a sample of tumor tissue or bodily fluid and sends it to a laboratory, where various methods are used to measure the level or presence (or absence) of the tumor marker.

2. If the tumor marker is being used to determine whether treatment is working or whether there is a recurrence, the marker's level will be measured in multiple samples taken at different times during and after treatment. Usually "serial measurements," which show how the level of a marker is changing over time, are more meaningful than a single measurement.

3. Some markers, such as the presence or absence of a particular genetic alteration that makes a tumor a candidate for treatment with a specific targeted therapy, do not themselves change over time. However, the proportion of tumor cells that have that alteration may change during and after treatment.

04.Can tumor markers be used in cancer screening?

1. Because tumor markers can be used to predict the response of a tumor to treatment and for prognosis, researchers have hoped that they might also be useful in screening tests that aim to detect cancer early, before there are any symptoms.

2. However, although tumor markers are extremely useful for determining whether a tumor is responding to treatment or assessing whether it has recurred, no tumor marker identified to date is sufficiently sensitive (that is, able to correctly identify people who have the disease) or specific (that is, able to correctly identify people who do not have the disease) to screen for cancer.

3. For example, until recently, the prostate-specific antigen (PSA) test, which measures the level of PSA in the blood, was used routinely to screen men for prostate cancer. However, an increased PSA level can be caused by benign prostate conditions as well as by prostate cancer, and most men with an elevated PSA level do not have prostate cancer. Because results from clinical trials showed that PSA testing leads at best to only a small reduction in the number of prostate cancer deaths and can lead to overdiagnosis and overtreatment, the PSA test is no longer recommended for routine screening. Now it is often used to monitor men with a history of prostate cancer to see if their cancer has come back.

3.3 Microarray technology

01. Microarray technology is a powerful tool for the simultaneous analysis of the expression of thousands of genes in tissues, organs, or cells. DNA sequence information encoding RNA for specific genes is physically printed onto microarray 'chips,' thus allowing measurement of the abundance of each RNA molecule in a biological sample. Through the use of bioinformatics, clustering, and gene network analysis, this technology is beginning to allow the identification of regulatory mechanisms that are associated with functional developments of the bovine mammary gland during late pregnancy, lactation, and involution, as well as prepubertal development.

02. Use of genome-enabled technologies (microarrays, proteomics, gene silencing) will contribute to our understanding of regulatory points at each stage of mammary development and will provide new insights into, and opportunities for, enhancing mammary growth and efficiency of milk production.

03. Microarray technology is based on a differential hybridization strategy, in which all mRNA that is expressed in the cell is copied into cDNA using reverse transcriptase and then used to hybridize to known sequences of DNA oligonucleotides in microarray platforms. Probe-target hybridization is usually identified by detection of labeled targets.

04. This technology can be used to identify global changes in transcript expression of thousands of genes in a single experiment. However, noted gene expression imbalances between samples should be confirmed with more specific techniques, such as quantitative RT-PCR.

05. To date, only 5 studies employing microarray technology for evaluation of human pituitary tumors have been published. The comparison of pituitary adenomas to normal pituitary corroborated earlier described genes with roles in cell proliferation or tumorigenesis, and has identified new candidate genes. Dissimilarity in gene expression profile between the different tumor subtypes was noted, and only six genes with proven roles in cell proliferation or tumorigenesis were differentially expressed in all pituitary tumor subtypes compared to normal pituitary: GADD45B1, SAT1, ID1, VIM, IGBP5 and ZFP36L1.

06. These findings suggest that genes involved in pituitary tumor formation are largely cell-subtype specific. One study compared prolactinomas according to growth behavior, and identified PTTG, CCB1, AURKB, ASK and CENPE as genes involved in tumor proliferation, invasion and aggressiveness. Therefore, elucidation of global gene expression imbalances may help identify new mechanisms for pituitary tumors, with implications for the recognition and identification of possible new therapeutic targets.

07. Microarray technology is not a simple genetic ‘cash cow.’ As in almost any scientific experiment, the careful selection of appropriate samples and controls (or matched pairs of samples) is required for valid and reliable findings. The statistical menace of false-positive findings within a plethora of transcriptional data is one of the biggest obstacles in applications of this technology. To diminish the false-positive discovery rate as much as possible, the a priori inclusion of sufficient replicates is mandatory.

08. The functional characterization of specific glycan–protein interactions will therefore require high-throughput technologies for surveying all the possible ligands for a particular lectin or antibody and vice versa:

1. Glycan Microarrays

    1. Microarrays technologies have played an integral role in the high-throughput analysis of nucleic acids and proteins and are beginning to provide insight into glycan–protein interactions as well as the specificity of glycan-processing enzymes through the development of glycan microarrays (Figure 23a). Glycans are typically derivatized at their reducing end for immobilization on surfaces either by absorption or covalent chemistries. The methods that have been utilized to absorb glycans on surfaces include direct spotting on glass slides, neoglycoconjugates on membranes, biotinylated-glycans on avidin-coated plates, and fluorous-phase interactions.

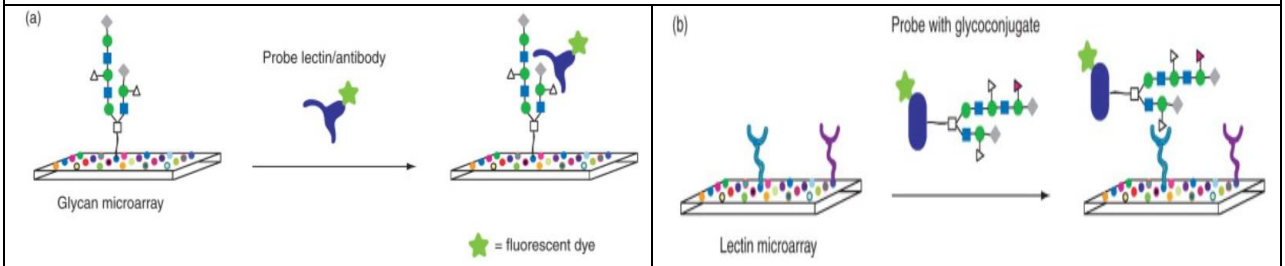
2. Alternatively, chemical strategies allow covalent immobilization of glycans through thiol, amine, and photolysis coupling methods. In addition, the 2,6-diaminopyridine derivatization of glycans is allowing multiplex analysis of glycan libraries by microarray, MS, and fluorescence imaging methods. Both naturally occurring and synthetic glycan libraries have been immobilized and used to interrogate the specificity of glycan-binding proteins, glycan-processing enzymes, and even whole cells.

2. Protein Microarrays: Lectins/Antibodies:

1. The high-throughput evaluation of glycan–protein interactions performs the reverse orientation with protein microarrays (Figure 23b). In this format, lectins or glycan-specific antibodies can be printed on glass slides by using protein microarray technologies and interrogated with fluorescently labeled glycoconjugates or cells (Figure 23b).

2. Indeed, several lectin or glycan-antibody microarrays have been developed to explore the glycosylation pattern of glycoproteins and cells. These studies have demonstrated that lectin microarrays can yield specific recognition of glycoconjugates, which upon further development should afford a useful means to rapidly survey glycosylation patterns of purified glycoconjugates and whole cells.

Figure 23. Microarray platforms for evaluating glycan–protein interactions



(a) Glycoconjugate microarrays (b) Lectin microarrays

3.4 Diagnosis of pathogenic microbes

01. Some infectious diseases are distinctive enough to be identified clinically. Most pathogens, however, can cause a wide spectrum of clinical syndromes in humans. Conversely, a single clinical syndrome may result from infection with any one of many pathogens. Influenza virus infection, for example, causes a wide variety of respiratory syndromes that cannot be distinguished clinically from those caused by streptococci, mycoplasmas, or more than 100 other viruses.

02. Most often, therefore, it is necessary to use microbiologic laboratory methods to identify a specific etiologic agent. Diagnostic medical microbiology is the discipline that identifies etiologic agents of disease. The job of the clinical microbiology laboratory is to test specimens from patients for microorganisms that are, or may be, a cause of the illness and to provide information (when appropriate) about the in vitro activity of antimicrobial drugs against the microorganisms identified (Fig. 10-1).

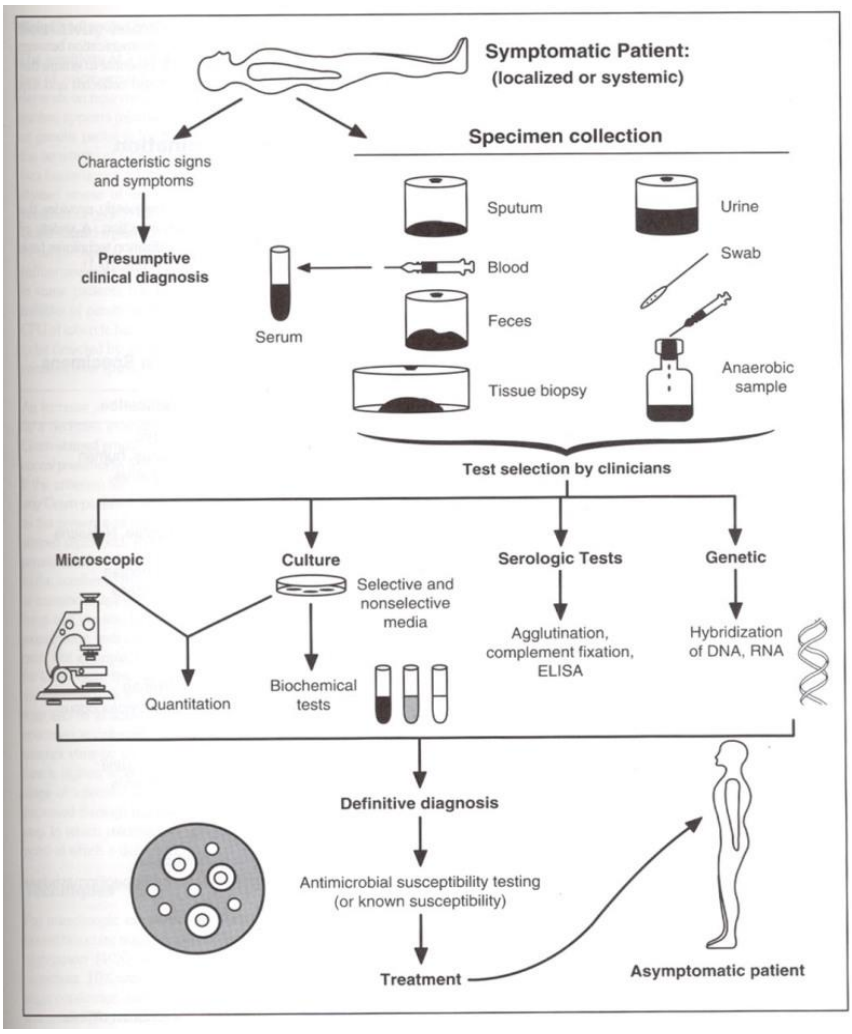


Figure 10-1: Laboratory procedures used in confirming a clinical diagnosis of infectious disease with a bacterial etiology.

03. The staff of a clinical microbiology laboratory should be qualified to advise the physician as well as process specimens. The physician should supply salient information about the patient, such as age and sex, tentative diagnosis or details of the clinical syndrome, date of onset, significant exposures, prior antibiotic therapy, immunologic status, and underlying conditions. The clinical microbiologist participates in decisions regarding the microbiologic diagnostic studies to be performed, the type and timing of specimens to be collected, and the conditions for their transportation and storage. Above all, the clinical microbiology laboratory, whenever appropriate, should provide an interpretation of laboratory results.

04. **Manifestations of Infection:** The manifestations of an infection depend on many factors, including the site of acquisition or entry of the microorganism; organ or system tropisms of the microorganism; microbial virulence; the age, sex, and immunologic status of the patient; underlying diseases or conditions; and the presence of implanted prosthetic devices or materials. The signs and symptoms of infection may be localized, or they may be systemic, with fever, chills, and hypotension. In some instances the manifestations of an infection are sufficiently characteristic to suggest the diagnosis; however, they are often nonspecific.

05.Microbial Causes of Infection:

1. Infections may be caused by bacteria (including mycobacteria, chlamydiae, mycoplasmas, and rickettsiae), viruses, fungi, or parasites. Infection may be endogenous or exogenous. In endogenous infections, the microorganism (usually a bacterium) is a component of the indigenous flora.

2. Endogenous infections can occur when the microorganism is aspirated from the upper to the lower respiratory tract or when it penetrates the skin or mucosal barrier as a result of trauma or surgery. In contrast, in exogenous infections, the microorganism is acquired from the environment (e.g., from soil or water) or from another person or an animal.

06.Specimen Selection, Collection and Processing:

1. Specimens selected for microbiologic examination should reflect the disease process and be collected in sufficient quantity to allow complete microbiologic examination. The number of microorganisms per milliliter of a body fluid or per gram of tissue is highly variable, ranging from less than 1 to 10^8 or 10^{10} colony-forming units (CFU). Swabs, although popular for specimen collection, frequently yield too small a specimen for accurate microbiologic examination and should be used only to collect material from the skin and mucous membranes.

2. Because skin and mucous membranes have a large and diverse indigenous flora, every effort must be made to minimize specimen contamination during collection. Contamination may be avoided by various means. The skin can be disinfected before aspirating or incising a lesion. Alternatively, the contaminated area may be bypassed altogether.

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Unit 4: Prevention and Treatment

4.1 Vaccines

01. Vaccines by definition are biological agents that elicit an immune response to a specific antigen derived from an infectious disease-causing pathogen. Edward Jenner developed the first vaccine in 1796 using cowpox to inoculate against smallpox. His groundbreaking work ultimately led to the global eradication of smallpox, officially declared in 1980. Since then, vaccines have helped to suppress the spread of several infectious diseases including polio, which has been eliminated from many countries, including all of those located within North and South America and Europe.

02. With the continued use of vaccines, it is tempting to speculate that other infectious diseases will soon follow suit. Unfortunately, we have taken a large step backward with the vocalization of the anti-vaccine movement and the reduction in vaccine acceptance.

03. Although arguably one of man's greatest discoveries, vaccines have always been met with some hesitation, even in the late 18th and early 19th centuries. Yet recently, vaccination has rapidly become a highly controversial issue, due in large part to an erroneous link between vaccines and autism. It is important to shed light on the necessity of immunization and the value it offers both personally and publicly.

04. Universal vaccine acceptance is essential to providing herd immunity, such that those who are unable to be directly protected by vaccination are protected by communal immunity. Vaccine education will be critical in

maintaining the forward progress that has been made in reducing or eliminating many infectious diseases. In an effort to ease some of these concerns, Federman suggests improved vaccine education as a public imperative in his perspective piece. He advocates that widely improving vaccine understanding will improve public perception of immunization and promote vaccine acceptance.

05. Lack of vaccine education and acceptance is one reason that many vaccines are under-utilized. One such vaccine is the influenza vaccine, which is one of the most complex and useful tools for preventing the spread of influenza. In this issue, both Lawrence and Murphy examine the lack of influenza vaccine coverage in at-risk populations, namely college students and pediatric asthma patients, respectively. Both suggest a lack of understanding as a barrier to proper vaccine acceptance.

06. Similar to the influenza vaccine, the hepatitis B vaccine lacks coverage in at-risk populations. Frew and colleagues examine the reasoning behind suboptimal immunization rates among Vietnamese Americans, an at-risk group for hepatitis B. Importantly, there is a vaccine available to combat this

disease, which is not always true for infectious diseases that often predominate in underprivileged populations. In his perspective, Erfe elaborates on the need for vaccines in developing nations and suggests a plan to mobilize pharmaceutical companies to research and produce vaccines for diseases such as Ebola, which have very little threat to the First World, but are ravaging sub-Saharan Africa and other vulnerable populations.

07. In addition to infectious disease-targeted vaccines, a recent endeavor has begun in the pursuit of anti-cancer vaccines. Similar to the foundation of infectious disease vaccines, anti-cancer vaccines stimulate an immune response to target cancer-specific antigens. In a comprehensive review, Liu describes the initial progress made and the potential for expanding the field by describing both therapeutic and preventive types of anti-cancer vaccines. In an equally thorough review, Datta and colleagues discuss the role of dendritic cells in cancer immunotherapy, not just in regard to the success of the FDA-approved sipuleucel-T for prostate cancer, but also the potential advancements for dendritic cell immunotherapy in other cancer treatment.

08. Conventional Vaccines

1. Classification:

1. Conventional vaccines originate from viruses or bacteria and can be divided in live attenuated vaccines and non-living vaccines. In addition, three vaccine generations can be distinguished for non-living vaccines.

    1. 1st generation vaccines consist of an inactivated suspension of the pathogenic microorganism. Little or no purification is applied.

    2. For 2nd generation vaccines purification steps are applied, varying from the purification of a pathogenic micro-organism (e.g., improved non-living polio vaccine) to the complete purification of the protective component (e.g., polysaccharide vaccines).

    3. 3rd generation vaccine are either a well-defined combination of protective components (e.g., acellular pertussis vaccine) or the protective component with the desired immunological properties (e.g., polysaccharides conjugated with carrier proteins).

2. An overview of the various groups of conventional vaccines and their generations is given in Table

Type	Example	Marketed	Characteristics[a]
Live			
Viral	Adenovirus	Yes	Oral vaccine, USA military services only, whole
	Poliovirus (Sabin)	Yes	Whole
	Hepatitis A virus	No	
	Measles virus	Yes	
	Mumps virus	Yes	
	Rubella virus	Yes	
	Varicella zoster virus	Yes	
	Vaccinia virus	Yes	
	Yellow fever virus	Yes	
	Rotavirus	No	
	Influenza virus	No	
Bacterial	Bacille Calmette–Guérin	Yes	Inactivated whole organism, oral vaccine
	Salmonella typhi	Yes	Inactivated whole
Non-living (first generation products)			
Viral	Poliovirus (Salk)	Yes	Purified inactivated whole
	Influenza virus	Yes	
	Japanese B encephalitis virus	Yes	
Bacterial	Bordetella pertussis	Yes	
	Vibrio cholerae	Yes	
	Salmonella typhi	Yes	
Non-living (second generation products)			
Viral	Poliovirus	Yes	
	Rabies virus	Yes	
	Hepatitis A virus	Yes	
	Influenza virus	Yes	Subunit vaccine
	Hepatitis B virus	Yes	Plasma-derived hepatitis B surface antigen
Bacterial	Bordetella pertussis	Yes	Bacterial protein extract
	Haemophilus influenzae type b	Yes	Capsular polysaccharides
	Neisseria meningitidis	Yes	Capsular polysaccharides
	Streptococcus pneumoniae	Yes	Capsular polysaccharides
	Vibrio cholerae	Yes	Bacterial suspension + B subunit of cholera toxin
	Corynebacterium diphtheriae	Yes	Diphtheria toxoid
	Clostridium tetani	Yes	Tetanus toxoid
Non-living (third generation products)			
Viral	Measles virus	No	Subunit vaccine, ISCOM formulation
Bacterial	Bordetella pertussis	Yes	Mixture of purified protein antigens
	Haemophilus influenzae type B	Yes	Polysaccharide-protein conjugates
	Neisseria meningitidis	No	Polysaccharide-protein conjugates
	Streptococcus pneumoniae	No	Polysaccharide-protein conjugates

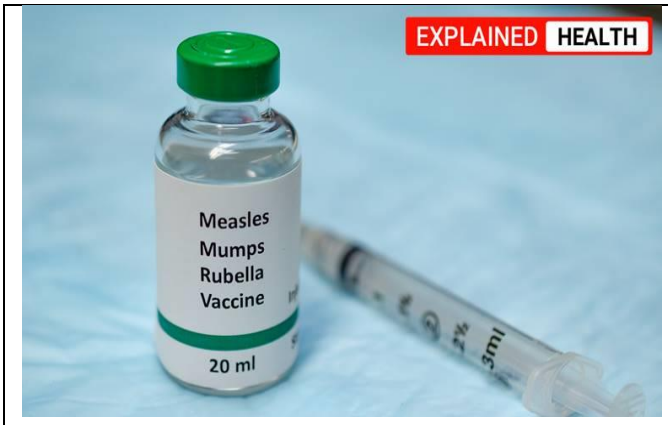
[a] Unless mentioned otherwise, the vaccine is administered parenterally.
Source: From Plotkin and Orenstein, 2004.

Table 4 Conventional vaccines.

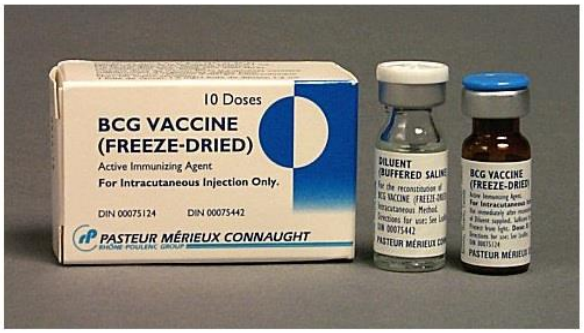
2. Live Attenuated Vaccines:

1. Before the introduction of recombinant-DNA (rDNA) technology, a first step to improved live vaccines was the attenuation of virulent microorganisms by serial passage and selection of mutant strains with reduced virulence or toxicity. Examples are vaccine strains for oral polio vaccine, measles-rubella-mumps (MMR) combination vaccine, and tuberculosis vaccine consisting of bacille Calmette-Gue´rin (BCG). An alternative approach is chemical mutagenesis. For instance, by treating Salmonella typhi with nitrosoguanidine, a mutant strain lacking some enzymes that are responsible for the virulence was isolated (Germanier and Fuer, 1975).

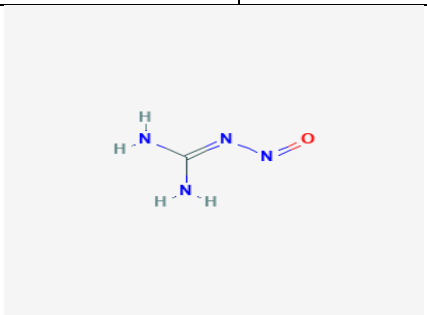
2. Live attenuated organisms have a number of advantages as vaccines over non-living vaccines. After administration, live vaccines may replicate in the host similar to their pathogenic counterparts. This confronts the host with a larger and more sustained dose of antigen, which means that few and low doses are required. In general, the vaccines give long-lasting humoral and cell-mediated immunity.



MMR Vaccine



BCG Vaccine



Nitrosoguanidine

3. Live vaccines also have drawbacks. Live viral vaccines bear the risk that the nucleic acid is incorporated into the host's genome. Moreover, reversion to a virulent form may occur, although this is unlikely when the attenuated seed strain contains several

mutations. Nevertheless, for diseases such as viral hepatitis, AIDS and cancer, this drawback makes the use of conventional live vaccines virtually unthinkable.

4. Furthermore, it is important to recognize that immunization of immunodeficient children with live organisms can lead to serious complications. For instance, a child with T-cell deficiency may become overwhelmed with BCG and die.

09.Recombinant Vaccines

1. Several genes from different etiologic agents have been cloned, expressed and purified to be tested as vaccines. There are a variety of expression systems for antigenic protein components, such as bacteria, yeast, mammalian cells and insect cells, in which the DNA encoding the antigenic determinant can be inserted and expressed. However, several factors must be taken into account before selecting the system for antigen expression.

2. The level of expression obtained using each specific expression vector and promoter, the selection marker of choice, the presence or absence of post-translational modification by the recombinant vector, among others, are essential features that interfere in the efficacy of production of recombinant antigens as vaccines. Bacterial expression systems are the most used due to the ease of handling and to their

capacity for high level expression. However, for antigens in which post-translational modifications (e.g., glycosylation) are necessary, the use of mammalian or insect cells should be considered.

3. Recombinant protein vaccines:

    1. Most of the vaccines under investigation today are based on highly purified recombinant proteins or subunits of pathogens. The classical example of recombinant protein vaccines currently in use in humans is the vaccine against hepatitis B (Table 1). Hepatitis B virus (HBV) infection is a chronic liver disease occurring worldwide.

    2. HBV presents a marked tropism for human liver cells, partially due to a specific receptor that is expressed on the surface of infected cells. The current vaccines are produced by expressing the hepatitis B surface antigen (HBsAg) in yeast cells. The HBsAg assembles into virus-like particles (VLPs), which are extremely immunogenic, making the HBV vaccine a very efficacious vaccine. The yeast expression system may secrete the antigen into the culture supernatant that can facilitate its purification.

    3. Furthermore, yeast cells offer some of the eukaryotic cellular machinery responsible for the post-translational modification of

proteins, being capable of rendering proteins glycosylated. The technology of production of the HBV vaccine has been transferred to several manufacturers and the prices have decreased due to competition, which has rendered this vaccine affordable to most developing countries.

Table 1.
Licensed[a] viral and bacterial vaccines for use in humans.

	Live attenuated	Killed inactivated	Subunit
Viral	Vaccinia	Polio (IPV)	Hepatitis B (HepB-surface antigen)
	Polio (OPV)	Rabies	Human papilloma virus (HPV)
	Yellow fever	Influenza	
	Measles	Hepatitis A	
	Mumps		
	Rubella		
	Influenza		
	Rotavirus		
Bacterial	BCG (tuberculosis)	*Bordetella pertussis* (whole cell)	Tetanus (toxoid)
	Salmonella typhi (oral)	Cholera	Diphtheria (toxoid)
		Bacillus anthracis	*Neisseria meningitidis* (polysaccharide)
			Bordetella pertussis (acellular)
			Streptococcus pneumoniae, 23 valent (polysaccharide)
			Haemophilus influenzae, type b (Hib) (polysaccharide)
			Neisseria meningitidis (polysaccharide conjugate)
			Streptococcus pneumoniae, heptavalent (conjugate polysaccharides)
			Salmonella typhi Vi (capsular polysaccharide)

[a]Licensed by national regulatory agencies such as ANVISA in Brazil or FDA in the USA.
OPV = oral polio vaccine; IPV = inactivated polio vaccine; BCG = bacillus Calmette-Guérin.

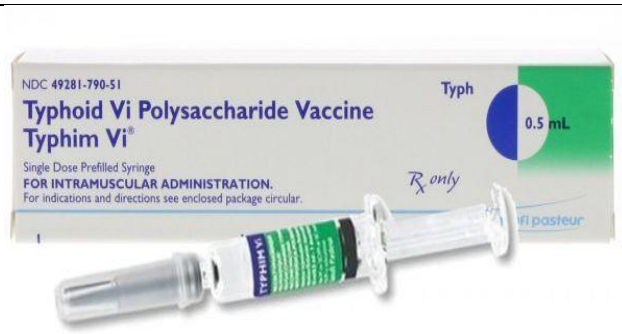
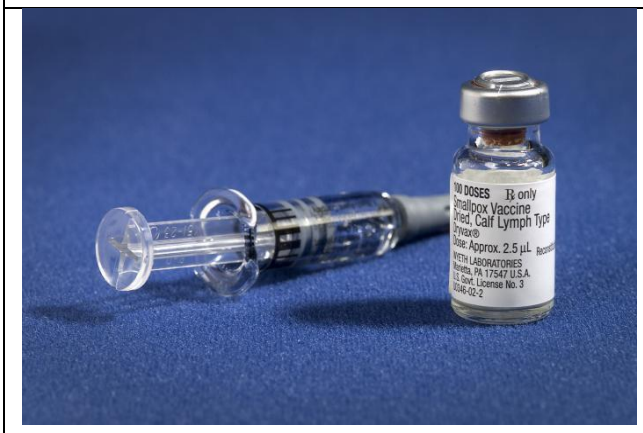
4. Live recombinant vaccines using bacterial or viral vectors:

    1. As a result of advances in the fields of molecular biology and genetic engineering it is now possible to create live recombinant vectors capable of delivering heterologous antigens by the introduction of antigen-encoding genes. The idea behind this approach is to use the capacity of infection and the immunological properties of the live vector to elicit an immune response against its own proteins, as well as towards the heterologous protein being presented.

    2. A number of bacteria [such as Salmonella typhi and bacille Calmette-Guérin (BCG)] and viruses [such as vaccinia (smallpox) and adenovirus] have been investigated as live recombinant vector vaccines. In general, these approaches have advantages that are intrinsic to the pathogen itself, such as mimicry of a natural infection, their capacity of stimulating both CD4+ and CD8+ T-cell subsets, and, in some cases, the possibility to be administered orally.

3. The use of live-attenuated bacterial vaccines is not novel. However, their utilization as carriers or delivery vehicles for heterologous antigen expression represents a technology with broad applicability that may have a significant impact on vaccine development. Significant advances in molecular biology have enabled precise deletions of genes encoding important virulence factors, as well as the introduction of recombinant DNA into avirulent yet immunogenic vaccine strains.

4. Bacterial vectors have many advantages that make them attractive systems for heterologous antigen presentation. They can elicit humoral and/or cellular immune responses and can be administered orally, thereby eliciting mucosal immunity. Most are antibiotic-sensitive strains, which allow antibiotic treatment if any adverse reaction occurs. In general, they display very favorable cost-effectiveness.

	
Mycobacterium bovis BCG vaccine	BCG Vaccine
	
Vaccinia (Smallpox) vaccine	Adenovirus vaccine

10.DNA Vaccines

1. DNA vaccination is a technique for protecting an organism against disease by injecting it with genetically engineered DNA to produce an immunological response. Nucleic acid vaccines are still experimental, and have been applied to a number of viral, bacterial and parasitic models of disease, as well as to several tumour models. DNA vaccines have a number of advantages over conventional vaccines, including the ability to induce a wider range of immune response types.

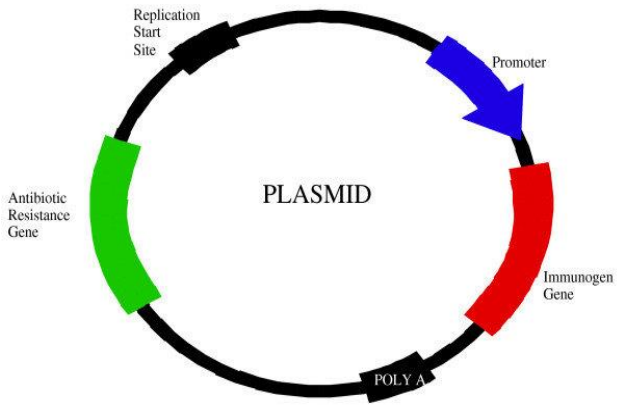
2. Vaccines are among the greatest achievements of modern medicine – in industrial nations, they have eliminated naturally-occurring cases of smallpox, and nearly eliminated polio, while other diseases, such as typhus, rotavirus, hepatitis A and B and others are well controlled. Conventional vaccines, however, only cover a small number of diseases, and infections that lack effective vaccines kill millions of people every year, with AIDS, hepatitis C and malaria being particularly common.

3. The vaccine DNA is injected into the cells of the body, where the "inner machinery" of the host cells "reads" the DNA and converts it into pathogenic proteins. Because these proteins are recognised as foreign, they are processed by the host cells and displayed on their surface, to alert the immune system, which then triggers a range of immune responses. These DNA vaccines developed from “failed” gene therapy experiments.

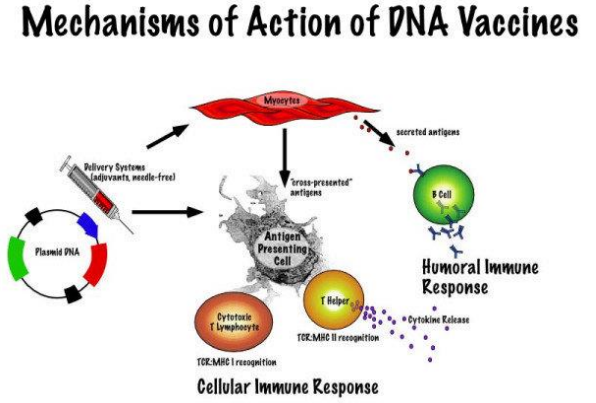
4. A DNA vaccine (or genetic vaccine as it is also called) consists of a plasmid containing:

    1. One origin of replication of Escherichia coli, for the amplification of the plasmid
    2. A strong promoter, generally from cytomegalovirus
    3. Multiple Cloning Sites, in which one can insert the gene to be expressed
    4. An antibiotic as selection marker

5. The first demonstration of a plasmid-induced immune response was when mice inoculated with a plasmid expressing human growth hormone elicited antibodies instead of altering growth. Thus far, few experimental trials have evoked a response sufficiently strong enough to protect against disease, and the usefulness of the technique, while tantalizing, remains to be conclusively proven in human trials. However, in June 2006 positive results were announced for a bird flu DNA vaccine and a veterinary DNA vaccine to protect horses from West Nile virus has been approved In August 2007, a preliminary study in DNA vaccination against multiple sclerosis was reported as being effective.



Components of DNA Vaccines



BCG Vaccine

6. Advantages and disadvantages of DNA vaccines are listed in the following slide

Advantages	Disadvantages
• Subunit vaccination with no risk for infection • Antigen presentation by both MHC class I and class II molecules • Able to polarise T-cell help toward type 1 or type 2 • Immune response focused only on antigen of interest • Ease of development and production • Stability of vaccine for storage and shipping • Cost-effectiveness • Obviates need for peptide synthesis, expression and purification of recombinant proteins and the use of toxic adjuvants ,Long-term persistence of immunogen • In vivo expression ensures protein more closely resembles normal eukaryotic structure, with accompanying post-translational modifications	• Limited to protein immunogens • Potential for atypical processing of bacterial and parasite proteins

4.2 Antibiotics

01. An antibiotic is a type of antimicrobial substance active against bacteria. It is the most important type of antibacterial agent for fighting bacterial infections, and antibiotic medications are widely used in the treatment and prevention of such infections. They may either kill or inhibit the growth of bacteria. A limited number of antibiotics also possess antiprotozoal activity. Antibiotics are not effective against viruses such as the common cold or influenza; drugs which inhibit viruses are termed antiviral drugs or antivirals rather than antibiotics.

02. Sometimes, the term antibiotic—literally "opposing life", from the Greek roots ἀντι anti, "against" and βίος bios, "life"—is broadly used to refer to any substance used against microbes, but in the usual medical usage, antibiotics (such as penicillin) are those produced naturally (by one microorganism fighting another), whereas nonantibiotic antibacterials (such as sulfonamides and antiseptics) are fully synthetic.

03. However, both classes have the same goal of killing or preventing the growth of microorganisms, and both are included in antimicrobial chemotherapy. "Antibacterials" include antiseptic drugs, antibacterial soaps, and chemical disinfectants, whereas antibiotics are an important class of antibacterials used more specifically in medicine and sometimes in livestock feed.

04. Antibiotics have been used since ancient times. Many civilizations used topical application of mouldy bread, with many references to its beneficial effects arising from ancient Egypt, Nubia, China, Serbia, Greece, and Rome. The first person to directly document the use of moulds to treat infections was John Parkinson (1567–1650). Antibiotics revolutionized medicine in the 20th century.

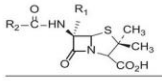
05.Penicillin:

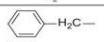
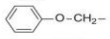
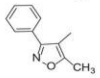
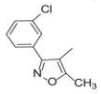
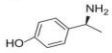
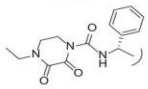
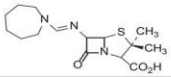
1. Penicillin G (benzylpenicillin) was the first β-lactam to be used clinically, most frequently to treat streptococcal infections for which it had high potency (Rammelkamp and Keefer 1943; Hirsh and Dowling 1946). Another naturally occurring penicillin, penicillin V (phenoxymethylpenicillin), in an oral formulation is still used therapeutically and prophylactically for mild to moderate infections caused by susceptible Streptococcus spp., including use in pediatric patients (Pottegard et al. 2015).

2. However, the selection of penicillin-resistant penicillinase-producing staphylococci in patients treated with penicillin G led to decreased use of this agent, and prompted the search for more penicillins with greater stability to the staphylococcal β-lactamases (Kirby 1944, 1945; Medeiros 1984).

3. A list of historically important and clinically useful penicillins is provided in Table 2. Among the penicillinase-stable penicillins of clinical significance are methicillin, oxacillin, cloxacillin, and nafcillin, with the latter suggested as the β-lactam of choice for skin infections, catheter infections, and bacteremia caused by methicillin-susceptible S. aureus (Bamberger and Boyd 2005). All were used primarily for staphylococcal infections until the emergence of methicillin-resistant S. aureus (MRSA) in 1979–1980 (Hemmer et al. 1979; Saroglou et al. 1980).

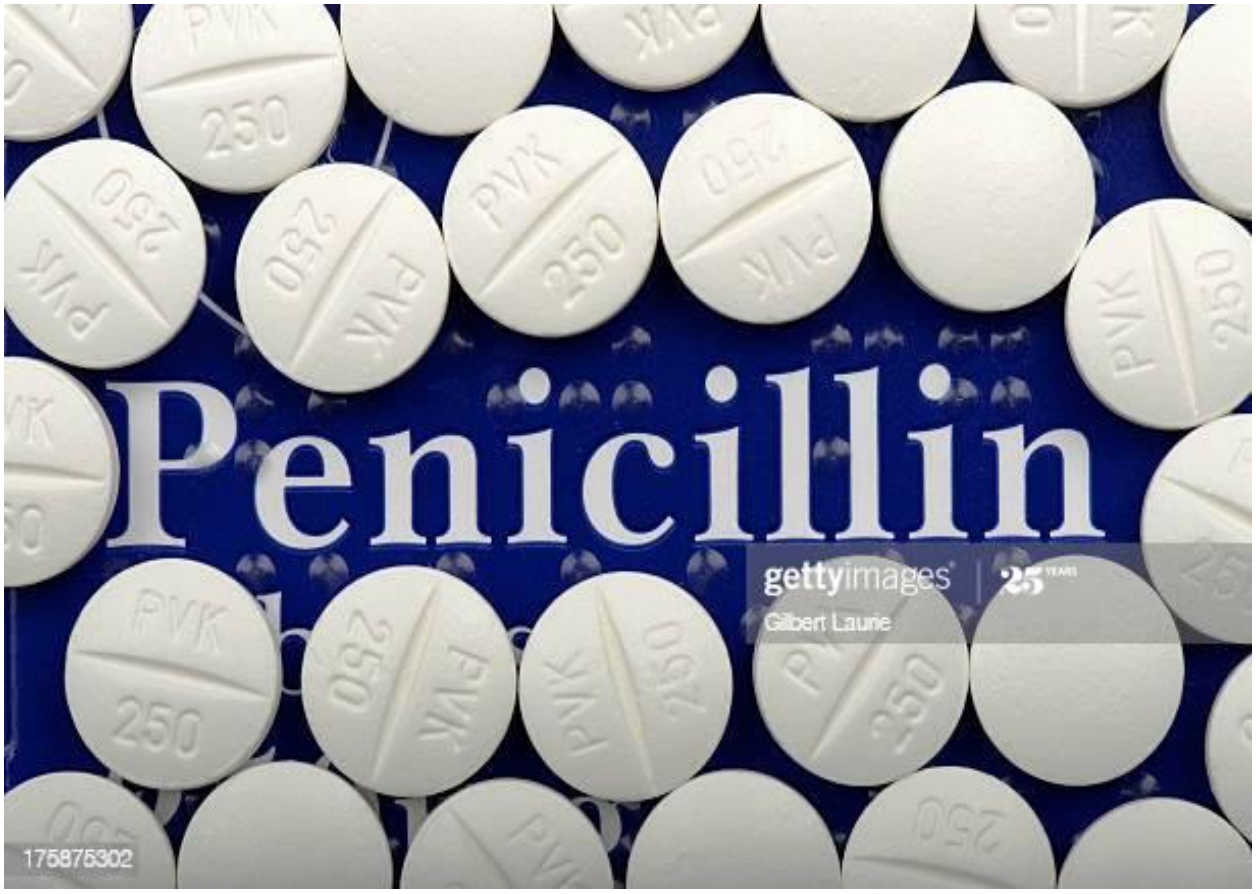
Table 2. Penicillins of current and historical utility



Name	R_1	R_2	Route of administration	Approval date[b,c]	Status
Benzylpenicillin (penicillin G)	—H		IM or IV	1946	Approved worldwide
Phenoxymethylpenicillin (penicillin V)	—H		Oral	1968	Approved worldwide
Methicillin	—H		IV	1960	No longer available; of historical interest
Oxacillin	—H		Oral, IV	1962	Widely available, but not in the United Kingdom
Cloxacillin	—H		Oral, IV	1974	Widely available, but not in the United Kingdom
Ampicillin	—H		Oral, IV	1963	Widely available
Nafcillin	—H		IV	1970	Limited availability
Amoxicillin	—H		Oral, IV	1972	Widely available
Carbenicillin	—H		Oral	1972	Discontinued
Ticarcillin	—H		IV	1976	Limited availability
Piperacillin	—H		IV	1981	Widely available, primarily in combination with tazobactam
Temocillin	—OCH_3		IV	1985 in Europe (Harvengt 1985)	Limited availability (Europe)
Mecillinam			IV	1978	Limited availability

IM, Intramuscular; IV, intravenous.
[a]FDA approval unless otherwise noted.
[b]Dates were updated from Medeiros (1997) (www.accessdata.fda.gov/scripts/cder/drugsatfda; www.drugs.com).

4. Penicillins with improved activity against Gram-negative pathogens included the orally bioavailable ampicillin and amoxicillin, both of which were introduced in the 1970s. These agents were initially used for the treatment of infections caused by *Enterobacteriaceae* and did not effectively inhibit the growth of *Pseudomonas aeruginosa*, which became more of a concern during the late 1970s.



06.Cephalosporin:

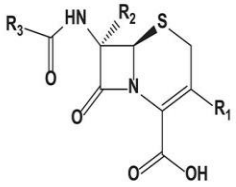
1. During the 1950s, the discovery of the naturally occurring penicillinase-stable cephalosporin C opened a new pathway to the development of hundreds of novel cephalosporins (Newton and Abraham 1956; Abraham 1987) to treat infections caused by the major penicillinase-producing pathogen of medical interest at that time, S. aureus. Dozens of cephalosporins were introduced into clinical practice (Abraham 1987), either as parenteral or oral agents.

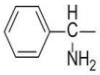
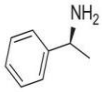
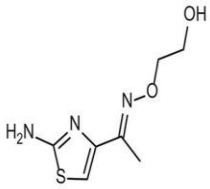
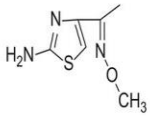
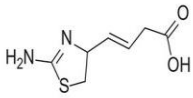
2. The molecules exhibited antibacterial activity with MICs often ≤4 μg/mL against not only staphylococci, but also Streptococcus pneumoniae and non-β-lactamase-producing enteric bacteria. The parenteral agents were generally eightfold more potent than the oral agents that were used in some cases to replace oral penicillins in penicillin-allergic patients.

3. The early cephalosporins, for example, those in the cephalosporin I subclass (Bryskier et al. 1994) introduced before 1980, were labile to hydrolysis by many β-lactamases that emerged following their introduction into clinical practice, so that only a few of the early molecules remain in use (see Table 3), primarily to treat mild to moderate skin infections caused by methicillin-susceptible S. aureus (MSSA) (Giordano et al. 2006).

4. Cefazolin with high biliary concentrations is still used for surgical prophylaxis and for treatment of abdominal infections (Sudo et al. 2014) and is effective as empiric therapy in 80% of Japanese children with their first upper urinary tract infection (Abe et al. 2016).

Table 3. Cephalosporins of current clinical utility or of historical interest



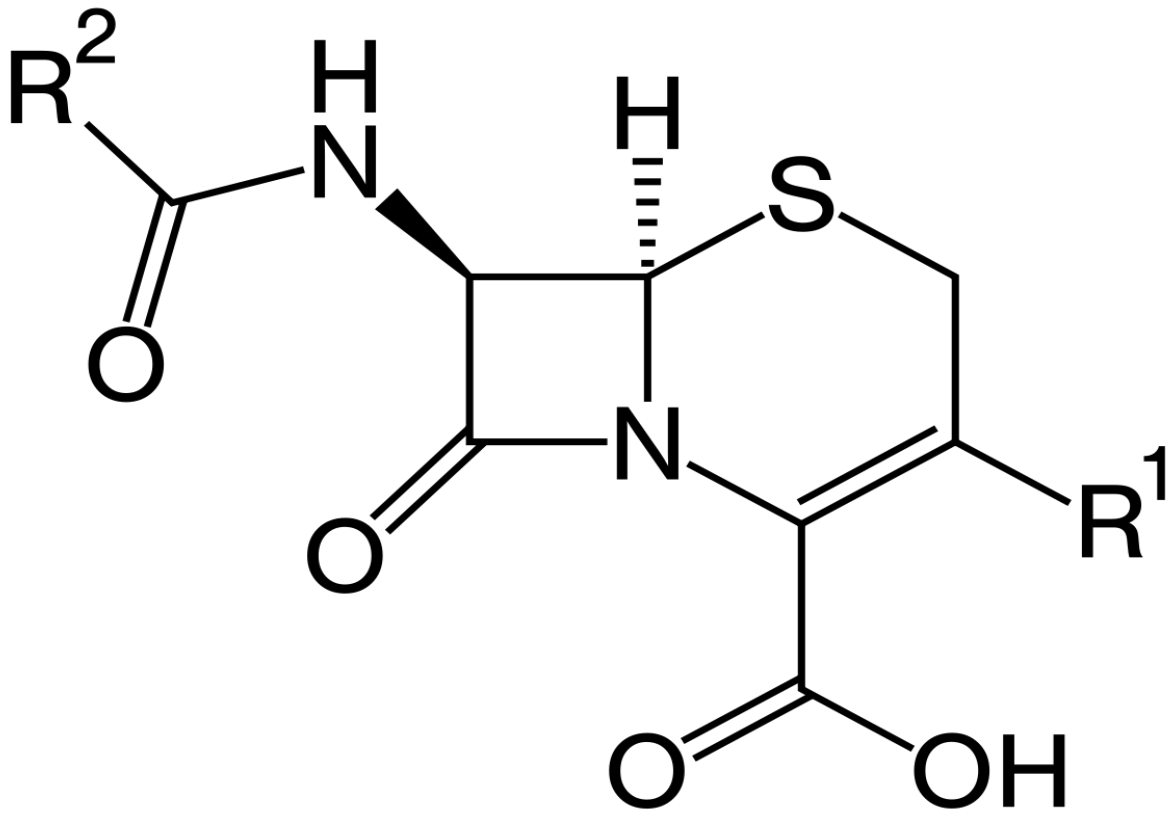
Name	Subclass[a]	R_1	R_2	R_3	Route of administration	Approval date[b,c]	Status
Cephalexin	Cephalosporin I		–H	$-CH_3$	Oral	1971	Limited availability
Cefaclor	Cephalosporin I	–Cl	–H		Oral	1979	Widely available
Cefixime	Cephalosporin V		–H		Oral	1989	Widely available
Cefpodoxime	Cephalosporin IV		–H		Oral	1992	Widely available
Ceftibutin	Cephalosporin III	–H	–H		Oral	1995	Widely available

5. When the TEM-1 penicillinase began to appear on transmissible plasmids in *Neisseria gonorrhoeae* (Ashford et al. 1976) and *Haemophilus influenzae* (Gunn et al. 1974; Khan et al. 1974), it was quickly recognized that the penicillins and cephalosporins in medical use were becoming ineffective, not only in treating those TEM-1-producing organisms, but also for the enteric bacteria and *P. aeruginosa* that could all acquire this enzyme. Another surge of synthetic activity in the pharmaceutical industry provided both oral and parenteral cephalosporins with stability to this common enzyme.

6. These agents tended to have decreased potency against the *staphylococci*, but gained antibacterial activity against Gram-negative pathogens. Cefuroxime, dosed parenterally or orally as the axetil ester, was the only member of the cephalosporin II class (Bryskier et al. 1994) with both oral and systemic dosage forms, but its stability to β-lactamase hydrolysis was diminished compared to later oral cephalosporins (Jacoby and Carreras 1990).

7. As seen with cefuroxime, acceptable oral bioavailability of cefpodoxime required esterification through addition of a proxetil group to attain sufficient absorption for efficacy (Bryskier and Belfiglio 1999). Of the oral agents approved after 1983 in Table 3, cefdinir was generally more stable to hydrolysis, not only to the original TEM enzyme, but also to the AmpC cephalosporinases that are produced at a basal level in many enteric bacteria and P. aeruginosa (Payne and Amyes 1993; Labia and Morand 1994).

8. Among the parenteral agents introduced in the 1980s were the cephamycin cefoxitin, and cephalosporins in the cephalosporin III and cephalosporin IV subclasses (Bryskier et al. 1994), which continue to serve as important antibiotics for the treatment of serious infections caused by Gram-negative pathogens.



SATHYABAMA
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SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY

UNIT – I - Medical Biotechnology – SBB3101

Subject Name: Medical Biotechnology Subject Code: SBB3101

SBB3101	MEDICAL BIOTECHNOLOGY	L	T	P	Credits	Total Marks
		4	0	0	4	100

COURSE OBJECTIVES

- To study about the medicinal approach of Biotechnology and recent advancements in diagnosis.

UNIT 1 ANIMAL CELL CULTURE
12 Hrs.

Animal cell culture-media, maintenance and culture of primary, secondary and continuous cell lines- organ culture-applications- cancer cell lines- apoptosis. Tissue Engineering – Skin, Liver, Pancreas. Assisted reproductive technology- Pregnancy diagnosis.

UNIT 2 CHROMOSOMAL ABNORMALITIES
12 Hrs.

Chromosomal disorders – Gene controlled diseases –Identification of disease genes Haemophilia, DMD, Alzheimer's – Molecular basis of human diseases: Pathogenic mutations – Oncogenes – Loss of function – Tumour Suppressor Genes Immunopathology: Hepatitis, Autoimmune Disorders.

UNIT 3 DIAGNOSTIC TECHNIQUES **12 Hrs.**

Prenatal diagnosis – Invasive techniques and Non-invasive techniques – Diagnosis of pathogenic microbes: Classical and modern methods- Diagnosis using protein and enzyme markers, DNA/RNA based diagnosis – Molecular markers – Microarray technology – genomic and cDNA arrays.

UNIT 4 PREVENTION AND TREATMENT **12 Hrs.**

Vaccines-conventional, recombinant, synthetic peptide, anti-idiotype, DNA vaccines Deletion mutant and vaccinia vector vaccine- Antibiotics-mode of action- antibacterial, antifungal, antiviral, antitumor antibiotics- synthetic chemotherapeutic agent development of microbial resistance to antibiotics.

UNIT 5 MODERN MEDICINE **12 Hrs.**

Hybridoma technique for MCAb production and applications- Gene therapy: Exvivo, In vivo, In situ- Cell and tissue engineering- Stem cell therapy- Nanomedicines- Gene products in medicine – Humulin, Erythropoietin, Growth Hormone/Somatostatin, tPA, Interferon.

Max Hours.60

TEXT / REFERENCE BOOKS:

1. Ramasamy, P. "Trends in Biotechnology", University of Madras, Pearl press, 2002. 2. Trevan. "Biotechnology". Tata McGraw-Hill, 2005.
2. Betty Forbes, Danial SAHM Alics Weinfield, Bailey 2007. Scott's diagnostic microbiology, 12th edition Mosby.
3. Jogdand, S. N. Medical Biotechnology, Himalaya Publishing house, Mumbai, 2005. 2. Click, B. R. and Pasternak. Molecular Biotechnology: Principle and applications of recombinant DNA. ASM Press, 2010.

END SEMESTER EXAMINATION QUESTION PAPER PATTERN

Max. Marks: 100 **Exam Duration: 3 Hrs.**
PART A: 10 questions of 2 marks each - No choice **20 Marks**
PART B: 2 questions from each unit of internal choice; each carrying 16 marks **80 Marks**

Unit 5: Prevention and Treatment

5.1 Hybridoma technique for MCAb production & its Applications

01. Hybridoma technology is a well-established method to produce monoclonal antibodies (mAbs) specific to antigens of interest. Hybridoma cell lines are formed via fusion between a short-lived antibody-producing B cell and an immortal myeloma cell. Each hybridoma constitutively expresses a large amount of one specific mAb, and favored hybridoma cell lines can be cryopreserved for long-lasting mAb production. As a result, researchers usually prefer generating hybridomas over other mAb production methods in order to maintain a convenient, never-ending supply of important mAbs.

02. Hybridoma technology was discovered in 1975 by two scientists, Georges Kohler and Cesar Milstein. They wanted to create immortal hybrid cells by fusing normal B cells from immunized mice with their myeloma cells. For incidental reasons, they had all the requirements fulfilled and it worked in the first attempt. By cloning individual hybrid cells, they established the first hybridoma cell lines which can produce single type of antibody specific to the specific antigen.

03. Their discovery is considered one of the greatest breakthroughs in the field of biotechnology. For the past decades, hybridomas have fueled the discovery and production of antibodies for a multitude of applications.

04. By utilizing hybridoma technology, Sino Biological provides cost-effective mouse monoclonal antibody service, and we can deliver you purified antibodies in 60 days.

05. The hybridoma technique has been successfully applied to produce MCAbs to red blood cell antigens for use in direct agglutination tests (Sonneborn et al. 1990; Sonneborn and Ernst 1987; Voak et al. 1981). However, in some cases, especially for protein antigens, production of IgM MCAbs to red blood cell surface antigens was not achieved (Ernst et al. 1999; Schmitz et al. 1996) and most of the obtained MCAbs were of the unexploited IgG isotype. In some other applications, the IgG MCAbs are preferred.

06. IgG MCAb has been demonstrated to have higher affinity than IgM. In addition, purification of IgG MCAbs is simpler than for the IgM isotype. However, in the conventional hybridoma technique, instead of obtaining the intended IgG MCAbs, some MCAbs of IgM isotype are always undecided obtained (Chiampanichayakul et al. 2006; Khunkaewla et al. 2007; our unpublished observations).

07. To overcome the problem of obtaining of unexpected isotype mAbs, in the present study we have modified the hybridoma technique for direct production of IgG or IgM mAbs against a desired antigen. Cells expressing IgG or IgM molecules were isolated from spleen cells of the immunized mice. The isolated cells were fused with myeloma cells using the standard hybridoma procedure. Using this approach, hybridomas producing IgG or IgM antibodies were obtained directly.

08.Steps Involved in Hybridoma Technology:

1. Cell fusion

    1. Polyethylene glycol (PEG) and electrofusion are commonly used to induce cell fusion in hybridoma production. PEG fuses the plasma membranes of adjacent myeloma and/or antibody-secreting cells, forming a single cell with two or more nuclei. This heterokaryon retains these nuclei until the nuclear membranes dissolve before mitosis.

    2. Electrofusion joins the membranes of neighboring cells by the application of a pulsed electrical field. Electrofusion is more efficient than PEG and the results are reproducible.

2. Hybridoma screening

    1. Even in the most efficient hybridoma fusions, only about 1% of the starting cells are fused, and only about 1 in 105 form viable hybrids. This leaves a large number of unfused cells still in culture. The cells from the immunized animal (antibody secreting cell) do not continue to grow in tissue culture and so do not confuse further work. However, the myeloma cells are well adapted to tissue culture and must be killed, which can be achieved by drug selection.

2. Commonly, the myeloma cells have a defective HGPRT enzyme (hypoxanthine-guanine phosphoribosyl transferase), blocking their ability to use the salvage pathway. These cells containing a non-functional HGPRT protein will die in HAT medium.

3. Only the hybridoma cells have got the ability to divide and proliferate on the HAT medium because genome from the B-lymphocyte makes them HGPRT positive and genome from the myeloma cells they can divide indefinitely.

4. HAT Medium (hypoxanthine-aminopterin-thymidine medium) is a selection medium for mammalian cell culture, which relies on the combination of aminopterin, a drug that acts as a powerful folate metabolism inhibitor by inhibiting dihydrofolate reductase, with hypoxanthine (a purine derivative) and thymidine (a deoxynucleoside) which are intermediates in DNA synthesis.

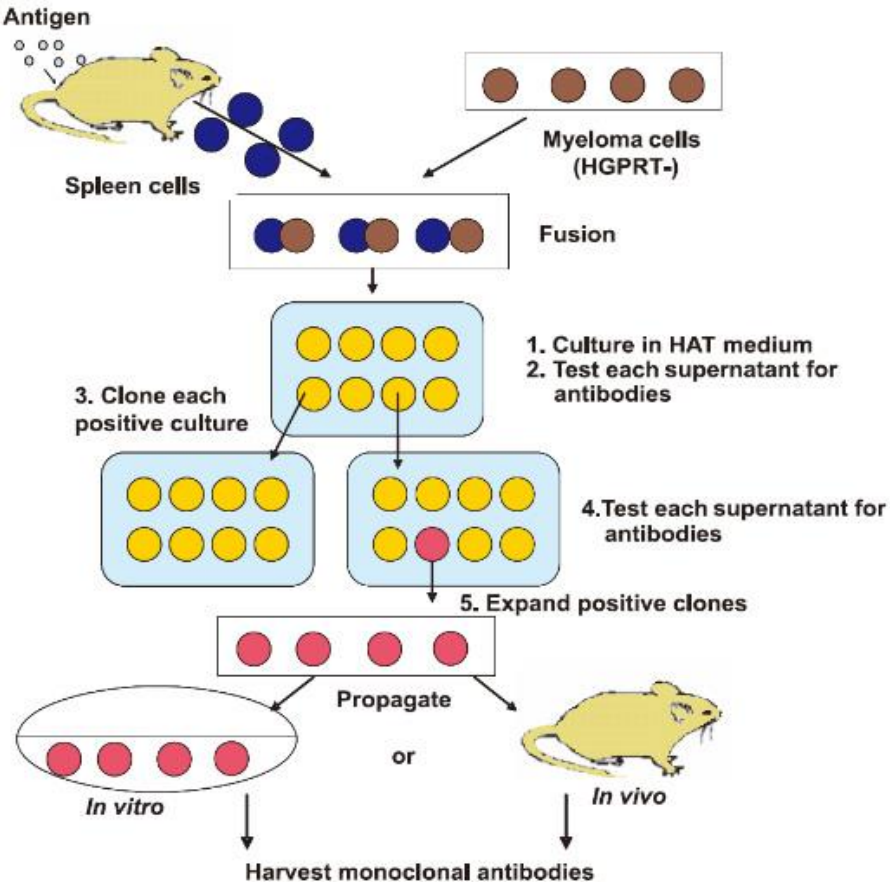
5. The trick is that aminopterin blocks DNA denovo synthesis, which is absolutely required for cell division to proceed, but hypoxanthine and thymidine provide cells with the raw material to evade the blockage (the "salvage pathway"), provided that they have the right enzymes, which means having functioning copies of the genes that encode them.

3. MCAb production

1. Hybridoma antibodies can be produced in vitro and in vivo.

2. For production of monoclonal antibodies in vitro, hybridomas are expanded by transfer to 24 well tissue culture plates followed by 25cm^2 flask and a 75cm^2 flask containing suitable medium. The cell density is maintained between 10^5 and 10^6 cells/ml. Typical culture supernatants yield up to 100μg/ml of antibody, the exact amount depending upon the cell density and rate of growth. Culture in vitro provides a more pure preparation of antibody. Sino Biological can offer serum-free hybridoma production serivce by the use of serum-free medium.

3. For producing monoclonal antibodies in vivo, mice are primed by intraperitoneal injection with 10^5 - 10^7 hybridoma cells. The rate of growth of the resulting ascites tumour is in general very variable and can be from less than two or more than five weeks. The ascites fluid can be collected from an anaesthetized mouse. It is possible to obtain 10 ml of ascites fluid or more from a mouse by regular tapping. Ascites fluid will be contaminated with mouse imunoglobulins to a small extent and if a very pure antibody is required this may prove inconvenient.


Antigen
Spleen cells
Myeloma cells
(HGPRT-)
Fusion
1. Culture in HAT medium
2. Test each supernatant for
antibodies
3. Clone each
positive culture
4.Test each supernatant for
antibodies
5. Expand positive clones
Propagate
or
In vitro
In vivo
Harvest monoclonal antibodies

5.2 Gene therapy

01. Gene therapy (also called human gene transfer) is a medical field which focuses on the utilization of the therapeutic delivery of nucleic acids into a patient's cells as a drug to treat disease. The first attempt at modifying human DNA was performed in 1980 by Martin Cline, but the first successful nuclear gene transfer in humans, approved by the National Institutes of Health, was performed in May 1989. The first therapeutic use of gene transfer as well as the first direct insertion of human DNA into the nuclear genome was performed by French Anderson in a trial starting in September 1990. It is thought to be able to cure many genetic disorders or treat them over time.

02. Between 1989 and December 2018, over 2,900 clinical trials were conducted, with more than half of them in phase I. As of 2017, Spark Therapeutics' Luxturna (RPE65 mutation-induced blindness) and Novartis' Kymriah (Chimeric antigen receptor T cell therapy) are the FDA's first approved gene therapies to enter the market.

03. Since that time, drugs such as Novartis' Zolgensma and Alnylam's Patisiran have also received FDA approval, in addition to other companies' gene therapy drugs. Most of these approaches utilize adeno-associated viruses (AAVs) and lentiviruses for performing gene insertions, in vivo and ex vivo, respectively. ASO / siRNA approaches such as those conducted by Alnylam and Ionis Pharmaceuticals require non-viral delivery systems, and utilize alternative mechanisms for trafficking to liver cells by way of GalNAc transporters.

04. The concept of gene therapy is to fix a genetic problem at its source. If, for instance, in an (usually recessively) inherited disease a mutation in a certain gene results in the production of a dysfunctional protein, gene therapy could be used to deliver a copy of this gene that does not contain the deleterious mutation, and thereby produces a functional protein. This strategy is referred to as gene replacement therapy and is employed to treat inherited retinal diseases.

05. Not all medical procedures that introduce alterations to a patient's genetic makeup can be considered gene therapy. Bone marrow transplantation and organ transplants in general have been found to introduce foreign DNA into patients. Gene therapy is defined by the precision of the procedure and the intention of direct therapeutic effect.

06. Somatic cell gene therapy involves the transfer of gene to a diseased somatic cell either within the body or outside the body with the help of a viral or non viral gene therapy vector.

07. Ex vivo is any procedure accomplished outside. In gene therapy clinical trials cells are modified in a variety of ways to correct the gene. In ex vivo cells are modified outside the patient’s body and the corrected version is transplanted back in to the patient. The cells are treated with either a viral or non viral gene therapy vector carrying the corrected copy of the gene.

08. Opposite of ex vivo is what we call in vivo where cells are treated inside the patient's body. The corrected copy of the genes is transferred into the body of the patient. The cells may be treated either with a viral or non viral vector carrying the corrected copy of the gene. If the patient is weak or the cell cannot be extracted out from the body, the gene is introduced directly into the body.

09. Gene therapy done in a restricted area or to a particular site is called in-situ. In situ gene therapy requires the vector to be placed directly into the affected tissues. In vivo gene therapy involves injecting the vector into the blood stream. The vector then must find the target tissue and deliver the therapeutic genes.

10.Methods of Gene Therapy:

1. In situ gene therapy:

    1. In situ gene therapy comprises transfer of corrected copy of the gene into the targeted organ or tissue. The major concern of current time gene therapy protocol is the lack of efficient transduction of the targeted organ. The method is effectively used against cystic fibrosis, a disease of airway epithelium.

    2. The method is also explored for cancer gene therapy where the viral vector is engineered to contain the herpes simplex virus thymidine kinase gene. After injection of the viral vector the patient is treated with a prodrug such as Ganciclovir, which causes 75% reduction in the tumor cell population.

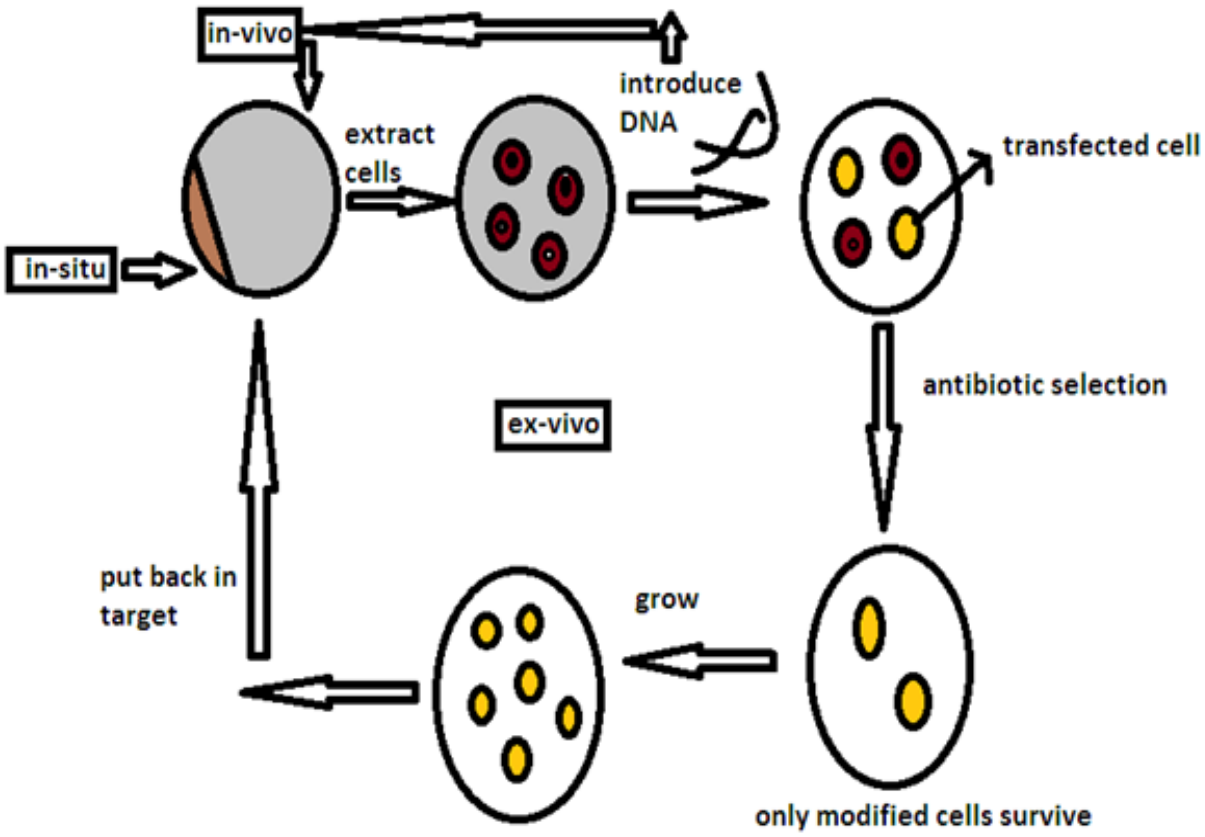
2. In vivo gene therapy:

    1. Delivery of corrected copy of the gene systemically through injection is a highly efficient way to transfer a transgene to the patient's body. The major problem of in vivo method is its inefficient targeting.

    2. The transgene delivered into the body by means of viral or non viral vector also evokes the immune response. The immune response against the vector leads to its clearance and only transient expression of transgene. The neutralizing antibody does not allow the second injection of the vector. Reducing the neutralizing antibody is the current area of research in order to improve the delivery of gene therapy vector.

    3. All gene therapy delivery protocols require the transgene to cross the plasma membrane and enter inside the nucleus. The major obstacle is still to deliver the transgene effectively to the intracellular compartment.

    4. Many modifications have been suggested into the viral vectors and also non viral vectors to target the gene to the tissue. VP22, a protein of herpes simplex virus has a property to spread from one cell to the other, and this property has been successfully implemented in designing the vectors.



Ex vivo, *in vivo*, and *in situ* gene therapy

5.3 Cell and tissue engineering

01. Tissue engineering is defined as *"the application of the principles and methods of engineering and life sciences toward the fundamental understanding of structure function relationships in normal and pathological mammalian tissue and the development of biological substitutes to restore, maintain or improve tissue function"* – Y.C. Fung.

02. *"The application of biological, chemical, and engineering principles toward the repair, restoration, or regeneration of living tissues using biomaterials, cells, and factors alone or in combination"* – C. T. Laurencin.

03. From these definitions, it is clear that tissue engineering has the potential to address the organ failure and tissue loss. This is because current clinical approaches for restoring the organ or tissue function are organ transplantation, surgical reconstruction, or use of prostheses. However, each treatment strategy has its own merits and demerits.

04. For example, organ transplantation is only treatment at the end stage of organ failure. This procedure involves mainly remove the failed organ from the patient and replaced with the procured organ for transplantation.

05. With today's medical advancement, it can be done for any organ – liver, kidney, heart, etc. But this whole procedure should follow legal and ethical considerations. Organ transplantation society encourages the use of organs donated from the cadavers instead from the living donors in order to reduce the risk for living donors while transplantation. Although such therapies have merit to save the lives of thousands, shortage of donor organs and tissues as the patient waiting list number has increased per year tremendously; and requirement of immunosuppressant limits its potential to address organ failure crisis.

06. In case of surgical reconstruction, there is no possibility of immune rejection because of the use of patient's own tissue (autologous). However, autologous grafting chiefly requires surgery at donor site; even limited supply, inadequate size and shape (Complaint mismatch) with donor site morbidity restrict its use towards tissue loss.

07. In some cases of organ failure, say for example loss of hand; or loss of leg; patients are advised to use artificial prostheses. But they are biologically non functional and they do not behave physiologically as a true organs. Examples are artificial heart, heart valves, prosthetic hip, and artificial breast. But these materials are subject to fracture, wear, toxicity, inflammation, which could induce the long term complications and rejections at the later stage. Hence limitations of existing therapies provoke the search of new technologies or therapies as tissue engineering to combat the organ failure and tissue lose crisis. Prof. Cato T. Laurencin defined Tissue Engineering as *"the application of biological, chemical, and engineering principles toward the repair, restoration, or regeneration of living tissues using biomaterials, cells, and factors alone or in combination"*.

5.4 Stem cell therapy

01. What is stem cell therapy?

1. Stem cell therapy is a form of regenerative medicine designed to repair damaged cells within the body by reducing inflammation and modulating the immune system. This phenomenon makes stem cell therapy a viable treatment option for a variety of medical conditions.

2. Stem cell therapies have been used to treat autoimmune diseases, orthopedic conditions, and traumatic injuries with studies conducted on use for Crohn's disease, Multiple Sclerosis, Lupus, COPD, Parkinson's, and more.

3. While stem cell therapy does not necessarily provide a cure for these conditions, the premise is to allow the body to heal itself well enough to mitigate the symptoms of the conditions for long periods. In many cases, this effect can substantially increase the quality of life for patients.

02.Where do stem cells come from?

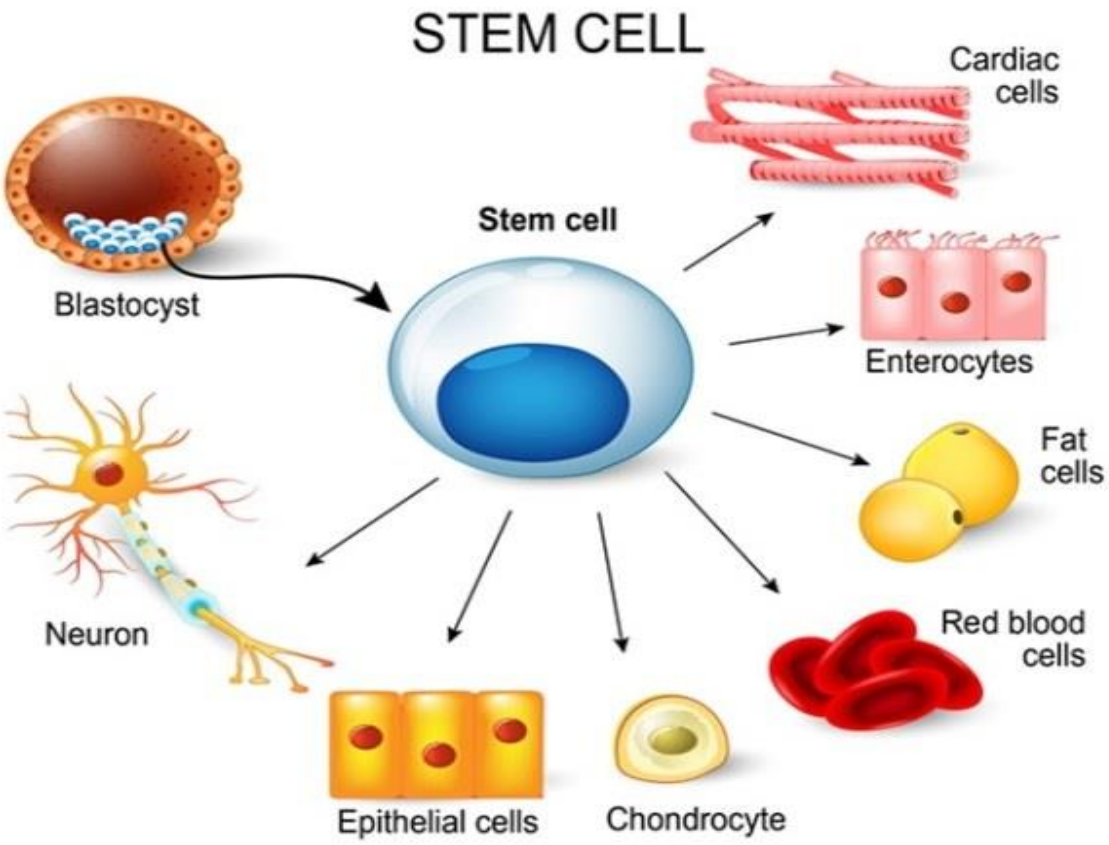
1. Stem cells can be obtained from many different sources. These include adipose (fat tissue), umbilical cord tissue, placental tissue, umbilical cord blood, or bone marrow.

03.How are stem cells administered?

1. Stem cells can be administered in a variety of fashions; IV Stem Cell Therapy (Intravenous administration), Intrathecal (directly into the spinal canal), Site injections into problem areas (Knee, hips, hands, etc.)

04.How does stem cell therapy work?

1. Stem cell therapy is a non-invasive treatment that aims to replace damaged cells within the body. Mesenchymal stem cell therapy can be deployed systemically via IV or injected locally to target specific sites, depending on patient needs.



Stem Cell Therapy

05. Stem cells target inflammation

1. The therapeutic uses of stem cells as a potential therapy for a variety of diseases has been immensely explored, the number of clinical trials conducted with Mesenchymal Stem Cells has increased exponentially over the past few years.

    1. MSCs are able to migrate and seed specifically into damaged tissue sites, where they can differentiate into functional cells to replace damaged or diseased cells.

2. Stem cells have a unique, intrinsic property that attracts them to inflammation in the body. Studies have shown that stem cells can regenerate damaged or diseased tissues, reduce inflammation and modulate the immune system promoting better health and quality of life.

3. Mesenchymal stem cells do this by influencing tissue repair via paracrine effects (cell signaling in order to change the behaviour of existing cells) or direct cell-to-cell contact.

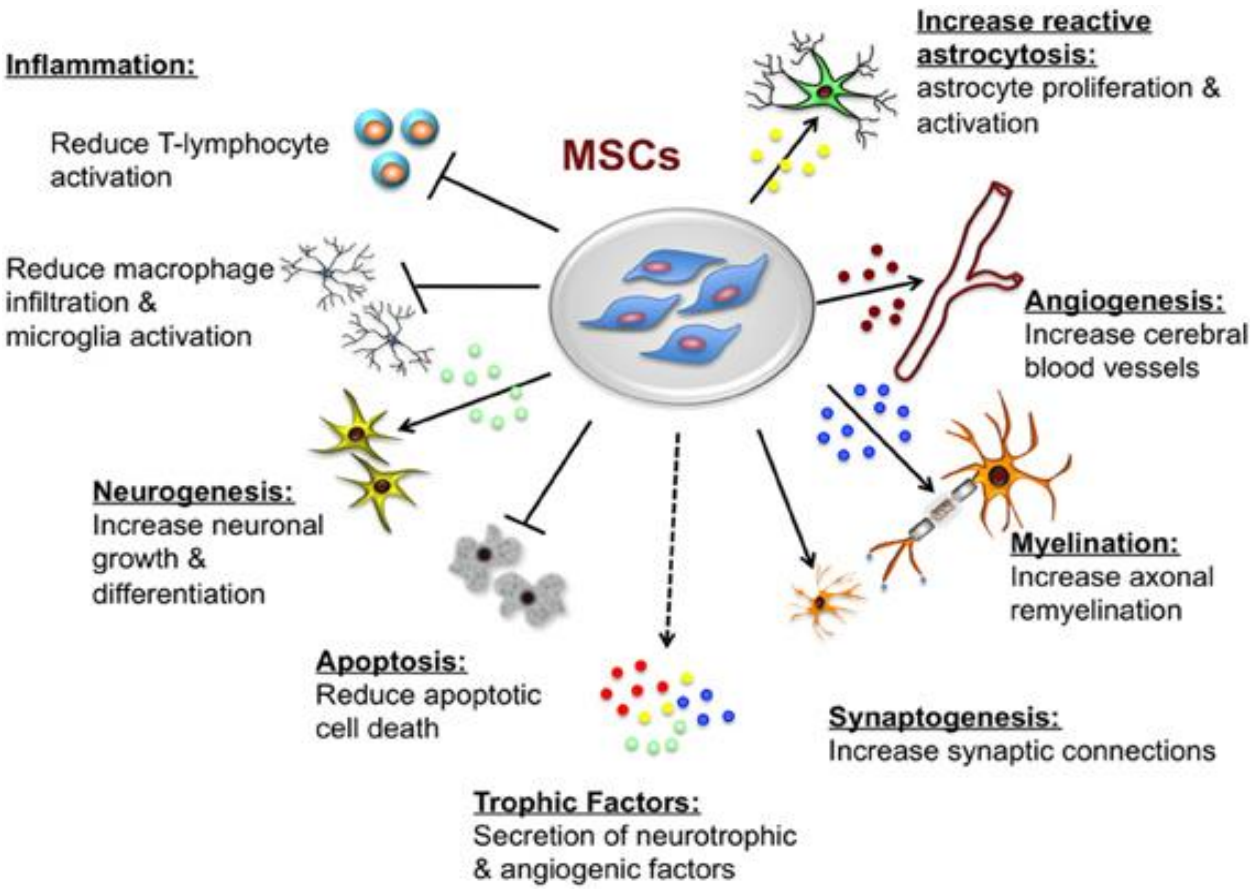


Diagram showing the processes of MSCs and how they reduce inflammation within the body

4. According to Mao F. et al.

    1. Mesenchymal stem cells (MSCs) facilitate tissue regeneration through mechanisms involving self-renewal and differentiation, supporting angiogenesis and tissue cell survival, and limiting inflammation.

5. What are stem cells?

    1. Stem cells are cells that have not yet specialized in the body, meaning they have not grown to a particular type of cell with a specific function (e.g. muscle cell, skin cell, etc.)

    2. According to Biehl et al., “The two defining characteristics of a stem cell are perpetual self-renewal and the ability to differentiate into a specialized adult cell type.”

6. Mesenchymal stem cells have the ability to differentiate:

    1. A stem cell can become many different cell types in the human body. The process of stem cells maturing into new types of cells is called differentiation. This process is the most critical aspect of stem cell therapies, as the cells become the type of cells required for one’s body to heal.

    2. Stem cells are also self-replicating; this ability allows the cells to multiply into identical copies of themselves. For example, if stem cells were used to treat a neurological injury, cells administered during treatment could become nerve cells, and then replicate to create exponentially more nerve cells on their own. This ability to duplicate drastically increases the effectiveness of stem cell treatments over time.

5.5 Gene products in medicine

01.Erythropoietin:

1. What is erythropoietin (EPO)?

    1. Erythropoietin (EPO) is a hormone produced by the kidney that promotes the formation of red blood cells by the bone marrow.

    2. The kidney cells that make erythropoietin are sensitive to low oxygen levels in the blood that travels through the kidney. These cells make and release erythropoietin when the oxygen level is too low. A low oxygen level may indicate a diminished number of red blood cells (anemia), or hemoglobin molecules that carry oxygen through the body.

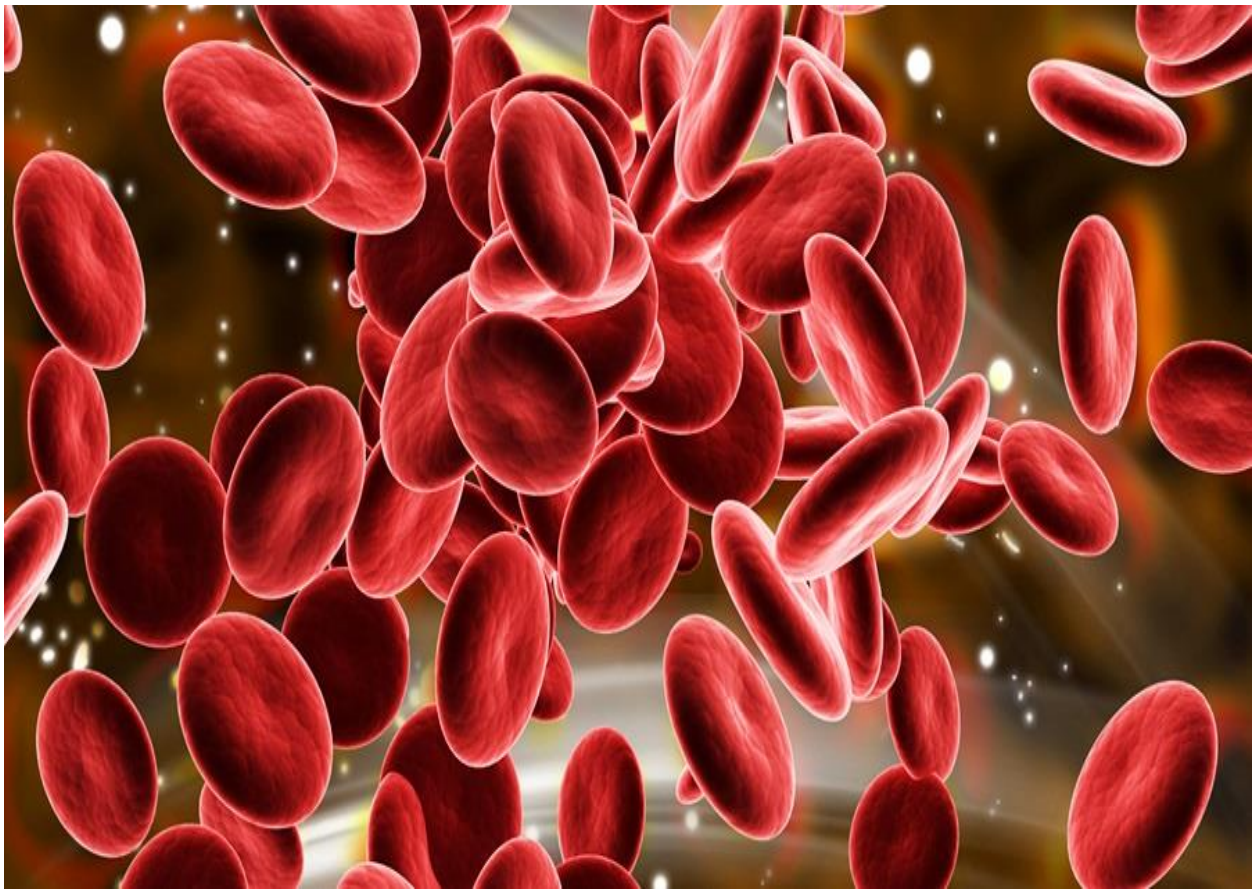
2. What does erythropoietin do? Why do we need it?

    1. Erythropoietin stimulates the bone marrow to produce more red blood cells. The resulting rise in red cells increases the oxygen-carrying capacity of the blood.

    2. As the prime regulator of red cell production, erythropoietin's major functions are to:

        1. Promote the development of red blood cells.

        2. Initiate the synthesis of hemoglobin, the molecule within red blood cells that transports oxygen.

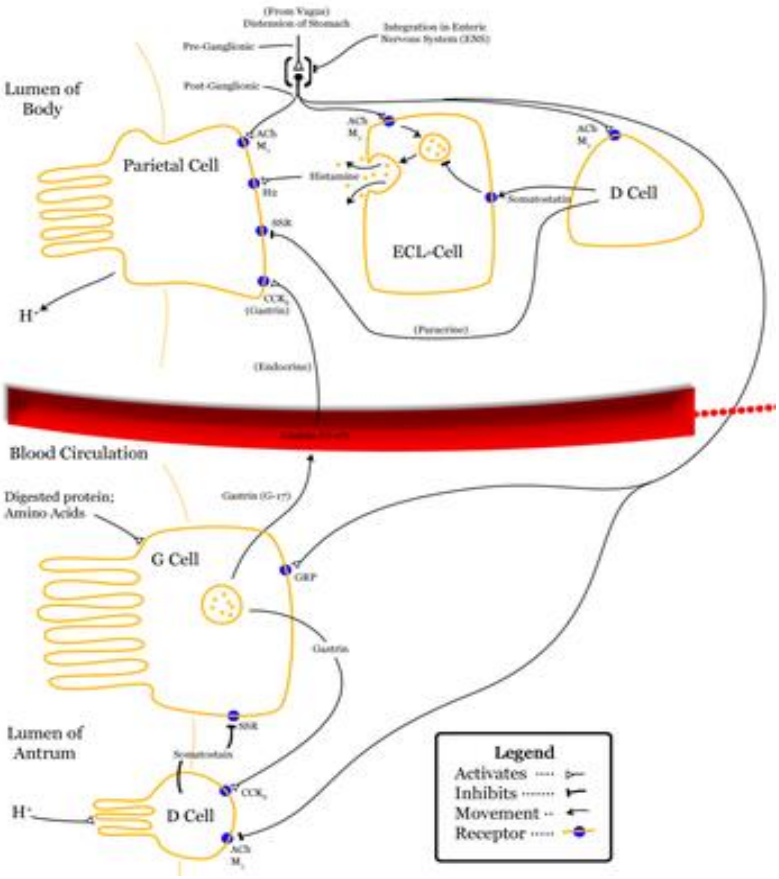


Erythropoietin

02.Somatostatin:

1. Somatostatin, polypeptide that inhibits the activity of certain pancreatic and gastrointestinal hormones. Somatostatin exists in two forms: one composed of 14 amino acids and a second composed of 28 amino acids. The name somatostatin, essentially meaning stagnation of a body, was coined when investigators found that an extract of hypothalamic tissues inhibited the release of growth hormone from the pituitary gland.

2. Somatostatin subsequently was found to be widely distributed throughout the central nervous system and to occur in other tissues.



Somatostatin