BIOTHERAPEUTICS AND STEM CELLS

Clinical importance of Therapeutic Proteins and Enzymes

- Proteins which are engineered in the laboratory for pharmaceutical use are referred to as **therapeutic proteins**
- Therapeutic proteins provide important therapies for a variety of diseases, such as diabetes, cancer, infectious diseases, hemophilia, and anemia.
- Common therapeutic proteins include antibodies, FC fusion proteins, hormones, interleukins, enzymes, and anticoagulants.
- Proteins which are absent or low in individuals with an illness such as Cancer, Infectious diseases, Hemophilia, Anemia, Multiple sclerosis, Hepatitis B/C, etc. are artificially synthesized on large scale through genetically modified host cells and delivered.
- This therapeutic approach in treating diseases using proteins and peptides is termed **protein therapeutics.**
- Protein therapy is similar to gene therapy, but unlike gene therapy, protein therapy delivers protein to the body in specific amounts (as would be ordinarily present), to help repair illness, treat pain or remake structures.
- **Introduced in 1920's Human insulin is considered to be the first therapeutic protein.**

SCOPE OF PROTEIN THERAPEUTICS:

- The **hope** is that the protein, which is not present in adequate levels, will function as it is designed to do.
- For example, use of certain proteins in addressing cardiovascular disease has been evaluated in some studies. Especially when veins or arteries become blocked, the right types of proteins might help here by building new passages for blood flow.
- Some doctors suggest that protein therapy of this type might eventually be so successful that it could eliminate the need for complicated surgeries like bypass surgery.

CLASSIFICATION:

Classification based on pharmacological action:

Group I: protein therapeutics with enzymatic or regulatory activity

a: Replacement of a protein that is deficient or abnormal: e.g. - Exubera, Increlex

b: Augmentation of an existing pathway: e.g. - Ovidrel , Neupogen

c: Provides a novel function or activity: e.g. - Myoblock

Group II : protein therapeutics with special targeting activity

a: Interferes with a molecule or organism: e.g. - Avastin

b: Delivers other compounds or proteins (such as radionuclie, cytotoxic drug or effector protein): e.g. – Ontak

Group III : Protein vaccines

a : Protecting against a deleterious foreign agent: e.g. - Engerix

b : Treating an autoimmune disease. : e.g. - Rophylac

Group IV : Protein diagnostics: e.g. – Geref

Classification based on molecular types:

- Antibody based drugs,
- Fc fusion proteins,
- anticoagulants,
- blood factors,
- growth factors,
- hormones,
- interferon,
- bone morphogenetic proteins,
- interleukins and
- thrombolytic.

Classification based on molecular mechanism:

- Binding non-covalently to target e.g. –mAbs
- Affecting covalent bonds e.g. – enzymes E
- xerting activity without specific interactions e.g. - serum albumin

Enzymes	Therapeutic Use	Basis	Additional Information
Prolactazyme	Lactose Intolerance	Prolactazyme is a proenzmye that produces lactase in stomach.	About 75% of the world's population is intolerant to lactose in adulthood. It occurs due to lack of lactase in digestive system.
Beta-Lactamase	Penicillin Allergy	Penicillin is converted to penicillioate	
Aglucerase	Gaucher's Disease type I	Enzyme replacement therapy	This disease is characterized by the lack of enzyme glucocerebrocidase.
Streptokinase	Heart Attacks (Myocardial Infarction)	Used as "clot blusters" to dissolve clots in the arteries of heart wall. Plasminogen is converted to plasmin which is fibrinolytic.	Administered i.v. to patients as soon as possible after the onset of a heart attack

Asparaginase	Acute Childhood Leukemia	Decreased level of serum asparagine and inhibition of aspargine dependent multiplication of tumor cells.	Tumor cells cannot synthesize L-asparagine due to lack of aspartate-ammonia ligase.
Collagenase	Skin ulcers	Causes collagen hydrolysis	Break up and remove dead skin and tissue
DNAse	Cystic Fibrosis (CF)	DNAse hydrolyses extracellular DNA responsible for Cystic Fibrosis.	DNA present in the mucous, which arises from dead WBCs and bacterial cells, serves to cross link the mucous, changing it from a fluid gel to a semi-solid.
Lysozyme	Antibiotic Therapy	Causes Bacterial cell wall hydrolysis	
Ribonuclease	Antiviral Therapy	Causes RNA hydrolysis	
Trypsin	Inflammation	Causes Protein hydrolysis	
Uricase	Gout	Converts Urate to allantoin	
Enzyme inhibitors	To increase the efficacy of drugs	Against resistant bacterisa	Example: Beta lactamase inhibitor

· **Top-selling therapeutic proteins**

Infliximab	Immune diseases
Trastuzumab	Cancer
Insulin glargine	Diabetes
Epoetin alfa	Anemia
Pegfilgrastim	Neutropenia
Ranibizumab	AMD
Darbepoetin alfa	Anemia
Interferon beta-1a (Avonex)	Multiple sclerosis
Interferon beta-1a	Multiple

Therapeutic monoclonal antibodies

Name	Type	Indication first approved
Muromonab-CD3	Anti-CD3; Murine IgG2a	Reversal of kidney transplant rejection
Abciximab	Anti-GPIIb/IIIa; Chimeric IgG1 Fab	Prevention of blood clots in angioplasty
Rituximab	Anti-CD20; Chimeric IgG1	Non-Hodgkin's lymphoma
Basiliximab	Anti-IL2R; Chimeric IgG1	Prevention of kidney transplant rejection
Daclizumab	Anti-IL2R; Humanized IgG1	Prevention of kidney transplant rejection
Palivizumab	Anti-RSV; Humanized IgG1	Prevention of respiratory syncytial virus infection
Infliximab	Anti-TNF; Chimeric IgG1	Crohn's disease
Trastuzumab	Anti-HER2; Humanized IgG1	Breast cancer

Therapeutic proteins are highly effective in vivo and have revolutionized treatment of diseases.

Protein therapeutics permits an individualized treatment approach by supporting a specifically targeted therapeutic process by compensating the deficiency of an essential protein.

Advantages of Protein therapeutics:

- A highly specific and complex set of functions
- Less potential to interfere with normal biological processes and cause adverse effects
- Well tolerated and less likely to elicit immune responses
- Effective replacement treatment without the need for gene therapy
- A faster clinical development and FDA approval
- Far-reaching patent protection

Hormones and Growth Factors used as therapeutics

(Erythropoietin & insulin as examples)

- Growth factors and hormones are signaling proteins that play a key role in cellular process such as growth, differentiation and signal transduction.
- Growth factors and hormones bind to various receptors on the target cell and induce signaling cascades that regulate physiological processes.
- **Growth factor**, any of a group of proteins that stimulate the growth of specific tissues. Growth factors play an important role in promoting cellular differentiation and cell division
- Some growth factors are similar to hormones in that they can be secreted into the blood stream, which carries them to their target tissues. However, whereas the production of hormones is limited to glandular tissue, growth factors can be produced by many different types of tissue.

There are **different kinds of growth factors**

- **Insulin-like growth factors (somatomedins)**, which stimulate growth by mediating the secretion of growth hormone from the pituitary gland
- **Epidermal growth factor (EGF):** stimulates the growth of epithelial cells
- **Keratinocyte growth factor (KGF):** Promotes the growth of keratinocytes, which secrete the protein keratin.
- **Transforming growth factors (TGF):** Promotes the growth of new blood vessels to create the proper supply of blood flow to a healing wound.
- **Vascular endothelial growth factor (VEGF):** Encourages the growth of angiogenesis, or new blood vessels.
- **Platelet-derived growth factor (PDGF):** Attracts fibroblasts and macrophages to the area of injured tissue
- **Nerve growth factor**, which stimulates the growth of neuronal cells.

Several growth factors are used **therapeutically.**

- **Erythropoietin**, which stimulates the growth of red blood cells, is used to treat anemia associated with chronic kidney failure, cancer chemotherapy, and zidovudine (AZT) therapy in AIDS patients.
- **Granulocyte colony-stimulating factor** (G-CSF; filgrastim) and **granulocyte-macrophage colony-stimulating factor** (GM-CSF; sargramostim)
    - used to stimulate the production of white blood cells in patients with cancer.
    - also can be used to mobilize hematopoietic progenitor cells (hematopoietic stem cells) into the peripheral blood circulation in order to generate cells that can be harvested and used for autologous bone marrow transplant

Administering Growth Factor Therapy

Growth factor therapy is most effectively administered through an injection that's minimally invasive and yields quick results.

Sometimes growth therapy can be delivered through a cream or gel that's applied to the wound, incorporated into wound dressings, or through skin grafts.

Growth factor therapy can be **used to treat a number of different conditions**, including:

- Arthritis
- Tendinitis
- Cartilage defects
- Back and neck pain
- Carpal Tunnel Syndrome
- Knee ligament injuries
- Plantar Fasciitis
- Sacroiliac joint pain
- Tennis elbow

Advantages of Growth Factor Therapy

Growth factor therapy uses the body's own cells to promote a more natural form of healing and less reliance on artificial agents. Some of the additional benefits include:

- Speeding up the time needed for the body to heal and for pain to subside
- Reduction of discomfort or disability for the patient
- Minimal side effects and complications are rare
- May be combined with other forms of treatment

Growth factor: Erythropoietin

- Erythropoietin is commonly referred to as hematopoietin or hemopoietin. It is also known in its abbreviated form of EPO.
- This is a glycoprotein cytokine that's primarily secreted by the kidney. This secretion takes place as the result of cellular hypoxia. This is a condition in which a part of the body doesn't receive as much oxygen as it needs.
- The most common causes of hypoxia in cells is anemia. As a result, people suffering from anemia are found to have increased levels of EPO.
- The consequence of EPO secretion is the stimulation of red blood cell production in bone marrow.
- Levels of EPO in the blood are naturally very low, unless anemia is present. It is thought that the production of EPO can multiply by 1000 when cells are suffering from anemia.

- Erythropoietin in blood is mainly of renal origin, with a small amount derived from the liver.
- The human erythropoietin gene is situated at chromosome 7q11-22, consisting of five exons and four introns, which produces a post-transcriptional single polypeptide containing 193 amino acids.

Function

- The primary function of erythropoietin is to produce more red blood cells. If bodies lack EPO, then the process in which red blood cells are produced cannot take place.
- EPO is found to have most of its effect in the bone marrow of humans, as this is where red blood cell precursors and progenitors are found. It works to protect these cells from death.
- Other functions include stimulating angiogenesis (the process in which new blood vessels are created from pre-existing ones), vasoconstriction-dependent hypertension, and improving cell survival through activating EPO receptors.

Erythropoietins available for use as therapeutic agents

- are produced by recombinant DNA technology in cell culture, and include Epogen/Procrit (epoetin alfa/ Erythropoietin alpha: RECOMBINANT HUMAN ERYTHROPOIETIN) and Aranesp (darbepoetin alfa);
- they are used in treating anemia resulting from chronic kidney disease, chemotherapy induced anemia in patients with cancer, inflammatory bowel disease (Crohn's disease and ulcerative colitis) and myelodysplasia from the treatment of cancer (chemotherapy and radiation).
- Adverse effects: increased risk of death, myocardial infarction, stroke, venous thromboembolism, and tumor recurrence

Hormones: Insulin

Insulin is a protein hormone that is used as a medication to treat high blood glucose.

- Insulin can be made from the pancreas of pigs or cows.Human versions can be made either by modifying pig versions or recombinant technology.
- Human insulin is in a class of medications called hormones.
- Human insulin is used to control blood sugar in people who have type 1 diabetes or in people who have type 2 diabetes that cannot be controlled with oral medications alone.
- Human insulin is used to take the place of insulin that is normally produced by the body. It works by helping move sugar from the blood into other body tissues where it is used for energy. It also stops the liver from producing more sugar.
- The types of insulin differ only in how quickly they begin to work and how long they continue to control blood sugar. (ultra short acting, short acting, intermediate acting, long acting)
- Human insulin comes as a solution (liquid) and a suspension (liquid with particles that will settle on standing). to be injected subcutaneously (under the skin). Human insulin is **usually injected subcutaneously** several times a day, and more than one type of insulin may be needed.
- Human insulin (Myxredlin, Humulin R U-100, Novolin R) solution **may also be injected intravenously** (into a vein) by a doctor
- The common side effect is low blood sugar. Other side effects may include pain or skin changes at the sites of injection, low blood potassium, and allergic reactions

Therapeutic use of Interferons

- **Interferons** play an **important role** in the innate immune response to virus infections
- Interferons are proteins that can induce a nonspecific resistance to viral infection by several mechanisms, including the inhibition of protein synthesis, inactivation of viral RNA, and enhancement of phagocytic and cytotoxic mechanisms.
- Interferon was named for its **ability to interfere** with viral proliferation.
- There are **three classifications of interferons on the basis of their antigenic specificities**--alpha (**α**), beta (***β***), and gamma (**γ**) interferon.
    - **IFN-α** is produced when leukocytes are infected with a virus. It is also called **leukocyte interferon**.
    - **IFN-*β*** is produced when fibroblasts are infected with a virus or treated with synthetic double-stranded RNA. It is also called **fibroblast interferon.**
    - **IFN-γ** is produced when lymphocytes are stimulated with a mitogen or sensitized lymphocytes are bound to an antigen. It is also called **immune interferon**.

Biological effects of interferons:

- **Effects on the immune system**: IFN has been known to have many effects on the immune system.
  - It generally **inhibit** antibody production and delayed-type (Type IV) hypersensitivity.
  - However, it **stimulates** cytotoxic T cells (killer T cells), NK cells, killer cells responsible for antibody-dependent cell-mediated cytotoxicity (ADCC),macrophages, and neutrophils
- **Effects on Viral Infections:** For the action mechanisms of IFN on viral infections
  - IFN directly provides antiviral effects and indirectly inhibits viral infections through the immune system
  - IFN enhances the activities of macrophages, NK, and ADCC to inhibit viral proliferation in infected cells and destroy infected cells.
- **Effect on tumours:** It is considered that the same mechanisms work for tumors.
  - IFN directly **inhibits the proliferation of tumor cells**, and generally has a stronger growth inhibitory effect on tumor cells than on normal cells.
  - IFN is also known to **induce apoptosis in some cells**. Thus, IFN not only directly inhibits the proliferation of tumor cells or destroys them, but also indirectly inhibits them by stimulating the immune system

Table 2 Various Biological Activities of IFN

1. Anti-tumor effect
2. Inhibitory effect on cell growth
3. Effects on lymphocytes
   a) Stimulation and inhibition of antibody production (B cell)
   b) Inhibition of delayed-type hypersensitivity (T cell)
   c) Inhibition of transplantation immune response (T cell)
   d) Inhibition of blastogenesis and DNA synthesis (T cell)
   e) Potentiation of killer T cells (T cell)
   f) Potentiation of natural killer activity (NK cell)
   g) Potentiation of ADCC activity
4. Effects on macrophages
   a) Potentiation of phagocytosis
   b) Potentiation of adherence to tumor cells
   c) Inhibition of intracellular bacterial proliferation
   d) MIF activity
   e) Chemotaxis
5. Other effects on cells
   a) Chemotaxis for neutrophils
   b) Potentiation of NBT reduction in neutrophils
   c) Increased histamine release in basophils
   d) Promotion of differentiation of erythroblasts
   e) Induction of differentiation of neuroblastoma cells
   f) Potentiation of expression of MHC Class I and II antigens

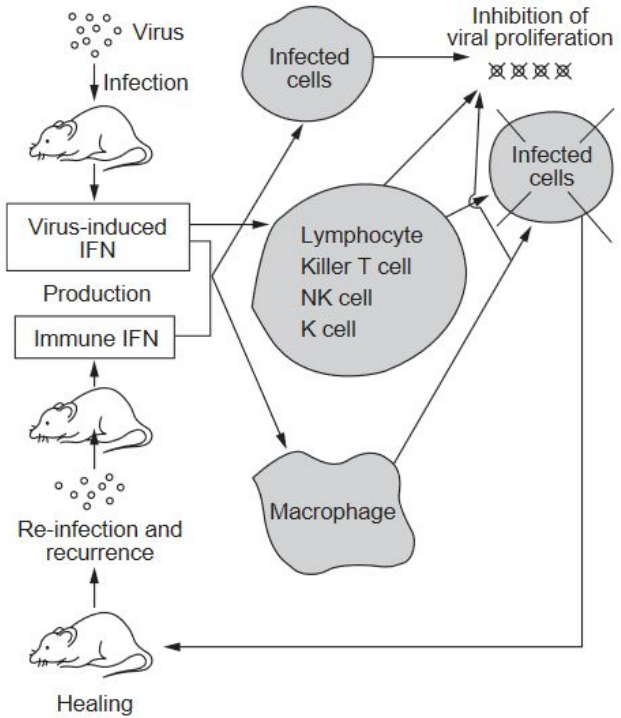


Fig. 3 Antiviral effect of IFN

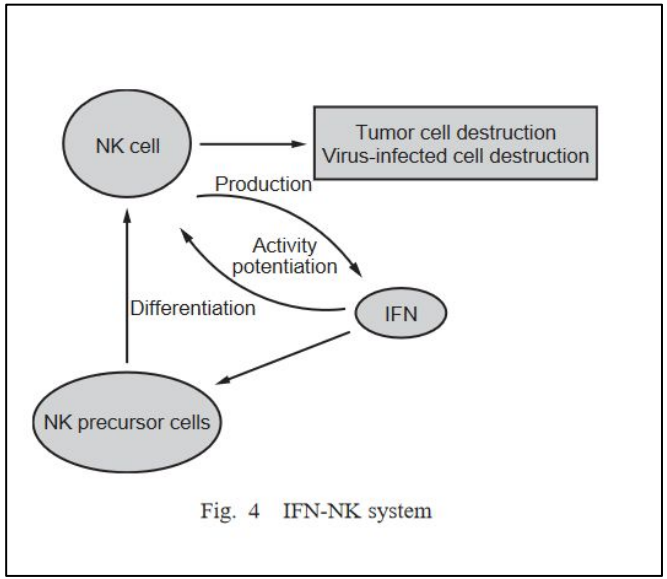


Fig. 4 IFN-NK system

- IFN is known to form a cycle with NK cells.
- That is, IFN increases NK activity, activated NK cells produce IFN, and IFN acts on NK precursor cells to induce the differentiation of NK cells, thereby increasing NK cells.
- This system formed by IFN and NK cells is called **IFN-NK system** (Fig. 4).
- The IFN-NK system is deeply involved in host defense against viral infection and against tumors.
- It is well known that the IFN-NK system strongly inhibits tumor metastasis

- IFN-alpha and IFN-beta were formerly called type I interferons
- IFN-gamma as type II

Therapeutic Applications

- IFNs are attractive biological response modifiers for use as therapeutic agents in infectious diseases, because they have **both antiviral and immunomodulatory activity.**
- IFNα is the most widely used interferon, and is produced commercially both by recombinant DNA technology and from stimulated leucocyte and lymphoblastoid cells. IFNβ is also available as a recombinant product and as a naturally produced product from human fibroblasts. IFNγ is available as a recombinant product.
- Interferons are therapeutically used in:
    - (a) **viral infections**, including viral hepatitis and human immunodeficiency virus infection;
    - (b) **neoplastic disorders**, including hairy cell leukaemia, multiple myeloma, cervical neoplasia, basal cell carcinoma, squamous cell carcinoma, melanoma, renal cell carcinoma, carcinoid tumours, cutaneous T cell lymphoma and non-Hodgkin's lymphoma;
    - (c) **myeloproliferative disorders**, including chronic myelogenous leukaemia and polycythaemia vera;
    - (d) **rheumatoid disorders**, including rheumatoid arthritis and systemic sclerosis;
    - (e) **other disorders**, including multiple sclerosis, chronic granulomatous disease and cryoglobulinaemia.

- Presently, there are the following **three main types of IFN-alpha preparations** that are commercially available.
    - Recombinant IFN-alpha2 preparations, which dominate the market are available in different forms of which IFN-alpha2b and IFN-alpha2a have undergone the most extensive clinical trials and are the most widely used.
    - Lymphoblastoid IFN-alpha contains a variety of IFN-alpha subtypes and is produced by lymphoblastoid cells that are grown in large tanks.
    - Leukocyte or natural IFN-alpha is produced by buffy coat cells derived from blood donors and stimulated by Sendai virus. These preparations are generally less well studied and the full potential of their therapeutic use has not been established as yet.
- **IFN-beta** has been available in a form produced by cultural human fibroblasts but today mostly recombinant forms
- **IFN-gamma** is available only as a recombinant substance.

Therapeutic use of interferons Side effects

- The common side effects of IFN treatment i.e. the "influenza-like" symptoms fever, chills, nausea fatigue, myalgia and loss of appetite- less severe with time and are usually tolerable
- Other side effects include mental depression which will prompt discontinuation of treatment, alopecia and weight loss- is associated with appearance of thyroid autoantibodies, usually disappears after cessation of IFN-alpha therapy.
- The risk for autoimmunity appears to be grater following treatment with IFN-gamma than with IFN-alpha

Therapeutic use of Interleukins

- **Interleukins** (ILs) are a group of cytokines (secreted proteins and signal molecules) that were first seen to be expressed by white blood cells (leukocytes) and mediate communication between the cells. Their major role is to create a stimulus for immune responses such as inflammatory conditions.
- The majority of interleukins are synthesized by helper CD4 T lymphocytes, as well as through monocytes, macrophages, and endothelial cells. They promote the development and differentiation of T and B lymphocytes, and hematopoietic cells.
- With interleukins (IL), a new class of potential drugs has been introduced into clinical research.
- These signal peptides are involved in the regulation of many physiological and pathophysiological processes.
- IL-1, -2, -3, -4, -6 and -11 have been tested in clinical trials.

Biological Activities of interleukins: The growth promoting, growth inhibiting or immunomodulatory

- ILs play essential roles in the activation and differentiation of immune cells, as well as proliferation, maturation, migration, and adhesion.
- They also have pro-inflammatory and anti-inflammatory properties.
- The primary function of interleukins is, therefore, to modulate growth, differentiation, and activation during inflammatory and immune responses.
- Interleukins consist of a large group of proteins that can elicit many reactions in cells and tissues by binding to high-affinity receptors in cell surfaces.
- They have both paracrine and autocrine function.

General Properties of Cytokines/Interleukins

- Cytokines are proteins made in response to pathogens and other antigens that regulate and mediate inflammatory and immune responses.
- Interleukin production is a self-limited process
- Interleukins have redundant functions. For instance, IL-4, IL-5, and IL-13 are B-cell growth factors and stimulate B-cell differentiation.
- Interleukins often influence other interleukin synthesis and actions. For instance, IL-1 promotes lymphocyte activation that leads to the release of IL-2.
- Cellular responses to cytokines are stimulated and regulated by external signals or high-affinity receptors. For example, stimulation of B-cells by pathogens leads to increased expression of cytokine receptors.
- Small quantities of a cytokine are needed to occupy receptors and elicit biologic effects.

Forty different types of interleukins are known, and they are designated numerically, IL-1 through IL-40

Functions of few Interleukins: https://www.ncbi.nlm.nih.gov/books/NBK499840/

ILs	SOURCE	Principal target	Action
IL-1	Macrophages, large granular lymphocytes, B cells, endothelium, fibroblasts, and astrocytes	T cells, B cells, macrophages, endothelium and tissue cells	lymphocyte activation, macrophage stimulation, increased leukocyte/endothelial adhesion,apoptosis in many cell types
IL-2	T cells	T cells	T-cell proliferation and differentiation, increased cytokine synthesis, potentiating Fas-mediated apoptosis, and promoting regulatory T cell development, proliferation and activation of NK cells and B-cell proliferation and antibody synthesis. Also, it stimulates the activation of cytotoxic lymphocytes and macrophages
IL-3	T cells and stem cells		functions as a multilineage colony-stimulating factor
IL-4	CD4+T cells (Th2)	B and T cells	It is a B-cell growth factor and causes IgE and IgG1 isotype selection. It causes Th2 differentiation and proliferation, and it inhibits IFN gamma-mediated activation on macrophages
IL-5	CD4+T cells (Th2).	B cells	It causes B-cell growth factor and differentiation and IgA selection. Besides, causes eosinophil activation and increased production of these innate immune cells.
IL-6	T and B lymphocytes, fibroblasts and macrophages	B lymphocytes and hepatocytes	B-cell differentiation and stimulation of acute phase proteins

Clinical Significance of interleukines:

- IL-1 acts on the hypothalamus to induce fever and is therefore called an endogenous pyrogen. It operates on hepatocytes to increase synthesis of specific serum proteins, such as amyloid A protein and fibrinogen. It causes fall in blood pressure or shock in large amounts. Corticosteroids inhibit the IL-1 effect.
- IL-12 overproduction causes allergic disorders. Corticosteroids inhibit the effects of IL-12
- The administration of IL-21 may be considered for use as a preventive and therapeutic approach when dealing with Th2-mediated allergic diseases.
- IL-26 shows high expression in psoriatic skin lesions, colonic lesions from individuals with inflammatory bowel disease and synovia of individuals with rheumatoid arthritis. It may constitute a promising target to treat chronic inflammatory disorders.
- IL-27 was found to exerts anti-inflammatory effects in several experimental autoimmune models. IL-27 treatment suppressed autoimmune diabetes.
- IL-28 may be sufficient treatment of HCV patients.
- IL-29 is a marker of osteoarthritis as joint inflammation implicates it
- IL-39 secreted by activated B cells may be a critical pro-inflammatory cytokine and a potential therapeutic target for the treatment of autoimmune diseases such as systemic lupus erythematosus
- IL-40 expression in several human B-cell lymphomas suggests that it may play a role in the pathogenesis of these diseases.
- In the **immunotherapy of melanoma and renal carcinoma** have successfully been used **interleukin-2 and interferon-gamma**. Their mechanisms of action involve the activation of natural killer (NK) cells and T lymphocytes. **The FDA has approved these 2 cytokines for the treatment of these 2 malignancies.**

Preservation and clinical use of blood and blood components

- The goal of **modern transfusion therapy** is to provide appropriate replacement therapy with blood components as opposed to whole blood for patients with specific hematologic deficiencies.
- A prerequisite of component therapy is, therefore, correct identification of the deficiency.
- Appropriate use of components avoids many of the hazards associated with the use of whole blood, and at the same time makes maximal use of this valuable resource.
- Hence it allows optimal transfusion therapy for each patient, but also fuller utilization of national blood resources.
- Blood components separated from whole blood soon after collection and appropriately stored can, in combination, provide all the factors present in fresh whole blood.

DEFINITIONS

Blood product — Any therapeutic substance prepared from human blood

Whole blood — Unseparated blood collected into an approved container containing an anticoagulant-preservative solution

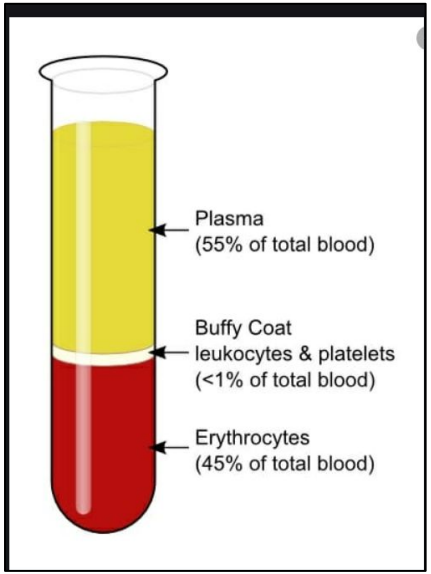
Blood component

1 A constituent of blood, separated from whole blood, such as:
- Red cell concentrate
- Red cell suspension
- Plasma
- Platelet concentrates

2 Plasma or platelets collected by apheresis [1]

3 Cryoprecipitate, prepared from fresh frozen plasma: rich in Factor VIII and fibrinogen

Plasma derivative [2] — Human plasma proteins prepared under pharmaceutical manufacturing conditions, such as:
- Albumin
- Coagulation factor concentrates
- Immunoglobulins



Apheresis: a method of collecting plasma or platelets directly from the donor,usually by a mechanical method

WHY THERE IS NEED FOR COMPONENT SEPERATION

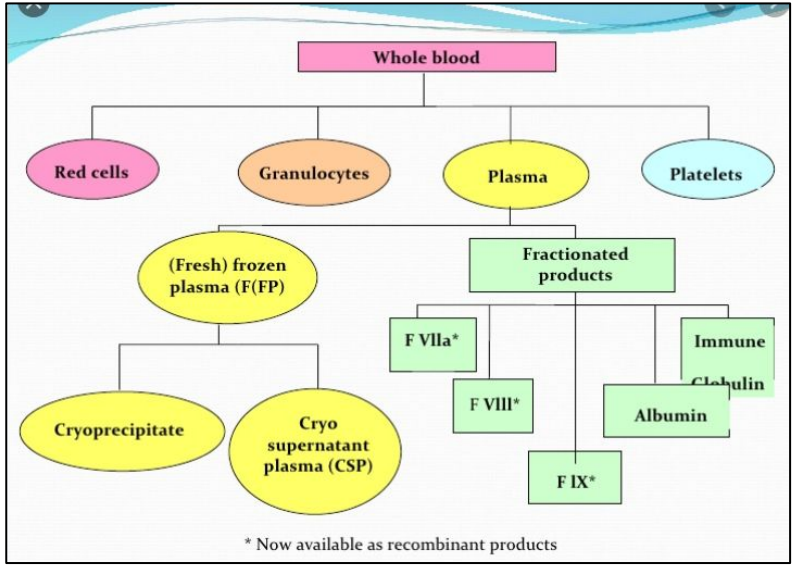
1. Separation of blood into components allows optimal survival of each constituents.

2. Component preparation allows transfusing only specific blood component that the patient requires.

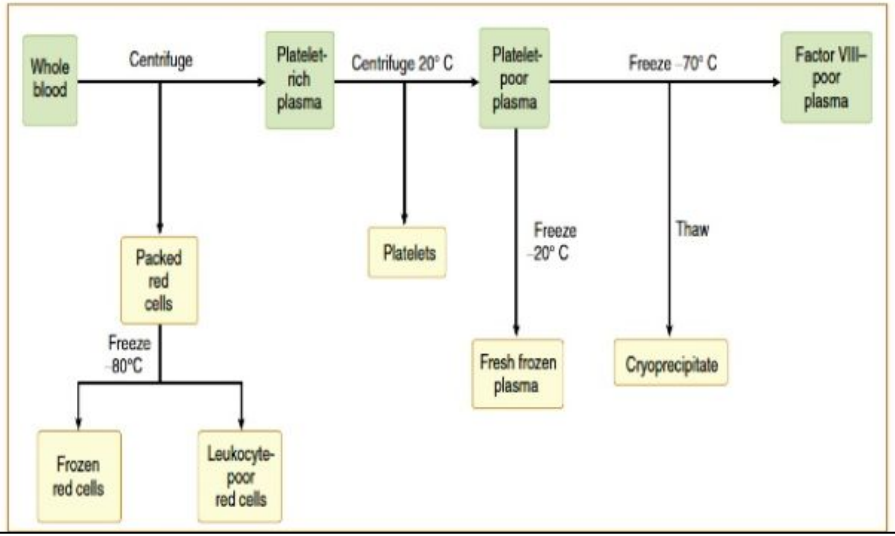
3. Transfusion of only the specific constituent of the blood needed avoids the use of unnecessary component, which could be contraindicated in a patient.

4. By using blood components, several patients can be treated with the blood from one donor, giving optimal use of every unit of donated blood.

5. Use of blood components, supplements blood supply - adds to blood inventory.

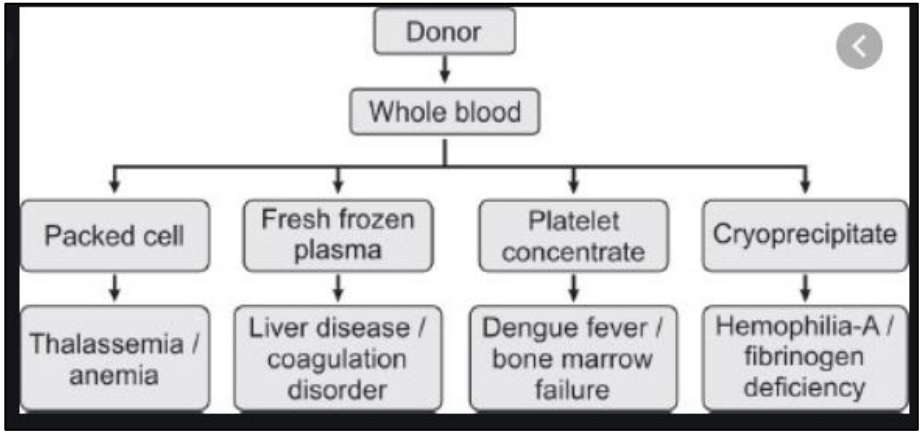


Scheme for separation of whole blood for component therapy



Component	Average Volume	Storage Parameters
Packed red blood cells	300 mL	1–6°C for 21–35 days or 42 days with additive solution
Red blood cells, frozen	300 mL	<–65°C for 10 years
Platelets, whole-blood-derived	50 mL per bag, usual dose 4–6 bags	20–24°C for 5 days
Platelets, apheresis	300 mL	20–24°C for 5 days
Plasma, fresh frozen	250 mL	<–18°C for 1 year or <–65°C for 7 years
Plasma, frozen within 24 h	250 mL	<–18°C for 1 year
Cryoprecipitate	15 mL per bag, usual dose 4–6 bags	<–18°C for 1 year

Clinical use of blood components:Blood component therapy



- Blood component transfusions are therapeutic and may be life-saving treatments for patients with a variety of conditions.
- Blood components (red blood cells, plasma, platelets and cryoprecipitate) are obtained by whole blood donation or by apheresis collection.
- Whole blood is collected from donors; however, the general practice is to process the whole blood into its components and then store these components under their optimal conditions. Apheresis instruments may also be used to collect blood components.
- Blood component therapy plays a critical role in the management of patients who are experiencing reduced hematopoiesis, increased peripheral destruction of cells, or generalized bleeding.
- The goal of blood component therapy is to achieve the desired effect without adverse outcomes.
- The desired effect may be to increase oxygen carrying capacity, correct coagulation factor deficiencies, or provide cellular components.
- However, adverse outcomes due to errors, accidents, or transfusion reactions cannot be completely avoid

- **Red cell** concentrates prepared from multiple packs have a hematocrit of approximately 70%. They may be stored for up to 3 weeks at 4 ℃ and are recommended for most situations requiring red cell transfusions.
- **Platelet** concentrates, which can be stored for up to 72 hours at 22 ℃, may be used for thrombocytopenic patients.
- **Fresh frozen plasma, stored plasma, cryoprecipitated factor VIII, factor VIII concentrate and factor IX complex concentrate** are available for the proper treatment of patients with hemorrhagic disorders due to coagulation factor deficiencies.
- Similarly, **albumin and immune serum globulin** are available for their oncotic and antibody properties respectively.
- Thus, the availability and appropriate use of the various blood products allows not only optimal transfusion therapy for each patient, but also fuller utilization of national blood resources.

Indications of blood component therapy:

- **RBC** transfusion is indicated for patients with acute blood loss experiencing hemorrhagic shock and may also be useful in patients with signs and symptoms consistent with symptomatic anemia.
- **Red blood cells** should be transfused only to increase oxygen-carrying capacity. Transfusion decisions should be based on individual patient physiology.
- **Platelet** transfusions are indicated for patients who are bleeding because of deficiency of platelets (thrombocytopenia) or the patient's platelets do not function properly (thrombocytopathy).
- The need for **platelets** during surgery can be predicted from the preoperative platelet count. Platelets are mobilized from the spleen and bone marrow as bleeding occurs.
- **Fresh frozen plasma (FFP)** is indicated for emergency reversal of warfarin, for correction of microvascular bleeding in the presence of elevated prothrombin time and partial thromboplastin time, and as part of a massive transfusion protocol
- **Cryoprecipitate** is a concentrated source of fibrinogen and selected coagulation factors. Cryoprecipitate may be more helpful in correcting the hypofibrinogenemia of dilutional or consumptive coagulopathy than fresh frozen plasma.

Adverse effects:

- Adverse reactions to blood components occur in 1% to 2% of transfusion episodes. Adherence to routine protocols for the evaluation of transfusion reactions may save lives.
- Acute hemolytic reactions are the leading cause of immediate transfusion fatalities. Prevention of these reactions requires strict adherence to transfusion and patient identification procedures.
- Transmission of infectious agents by transfusion has been markedly reduced, and bacterial infection is now the most common infectious complication of transfusion.
- **Informed consent for blood transfusion** is a standard of practice. A competent adult has the legal right to refuse blood transfusion. Consent in critically ill patients remains subject to individual institution policies.

Principles and safety guidelines for blood transfusion

- Blood transfusion refers to a large range of therapeutic products prepared in the blood bank from the whole blood or manufactured from pooled human plasma by selective procedures for the purpose of the clinical usage.
- The blood products include whole blood, packed Red Blood Cells, Fresh Frozen Plasma, Platelet Concentrates, Cryoprecipitate, Granulocytes, Leucoreduced Products, Irradiated Products and Plasma Derivatives.
- Blood transfusion has become well regulated through Guidelines by organizations such as American Association of Blood Banks (AABB), Council of Europe Health Institutions and Organization and World Health Organization (WHO).

The departmental and interdepartmental policies and procedures could be developed for :

- Requests for transfusion, collection of blood samples for pre-transfusion compatibility testing.
- Collection of blood and blood components from the Blood Bank and their delivery to the ward.
- The administration of blood and blood components
- Documentation of transfusions
- The care and monitoring of transfused patients
- The management and reporting of adverse events
- The specification of staff responsible for various tasks.

Request for Transfusion

1- Request for transfusion of blood/blood component is to be made by the physician on a Blood Bank request form and should includes

(a) Patient Identification :-

Surname , First name , Date of birth ,

Hospital number / accident .

(b) Type of blood component or blood product

(c) Specify product requirements (e.g. CMV negative , irradiated etc.)

(d) Number of units and/or volume of product or apheresis

(e) Unmatched blood (in emergency or life- threatening situations

2- Telephone orders are accepted in urgent situations but should be followed-up with a standard request.

Component Preparation and Selection

- Pre-transfusion compatibility testing must be performed for whole blood and red cell components prior to issue to minimize the risk of hemolytic transfusion reaction and maximize post-transfusion red cell survival.
- The majority of ABO incompatible transfusion due to documentation / identification error .
- Compatibility testing is not required for platelet and plasma components. However, ABO and Rh blood groups of the intended recipient must be known prior to component selection for issue.
- Components issued for transfusion must have been tested and found negative for markers of the following infective agents : Hepatitis B and C, Syphilis, HTLV, HIV and malaria parasites.

Compatibility testing

- Can be divided into 3 categories:
  - Preanalytical procedures
  - Serological testing
  - Postanalytical procedures

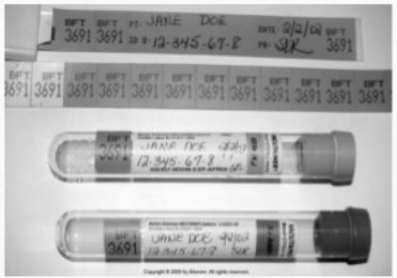
Pre-analytical phases

- Patient identification
- Specimen collection
- Review of patient history

Patient Identification

- Must confirm recipient's ID from bracelet ON the patient
  - Full patient name and hospital number
  - Name of physician

Sample Identification



- The sample should also have the full patient name, hospital number, and physician
- Date and time of collection, phlebotomist's initials
- All of this should be on the request form and the sample

Specimen Collection

- Collected in tube with EDTA and no additives
- If the venipuncture causes hemolysis, the sample may be rejected
- Samples are labeled at the bedside (pre-labeling is not recommended)
- A record of individuals who collect (or test) the specimens should be documented in order to "backtrack" in case of an error

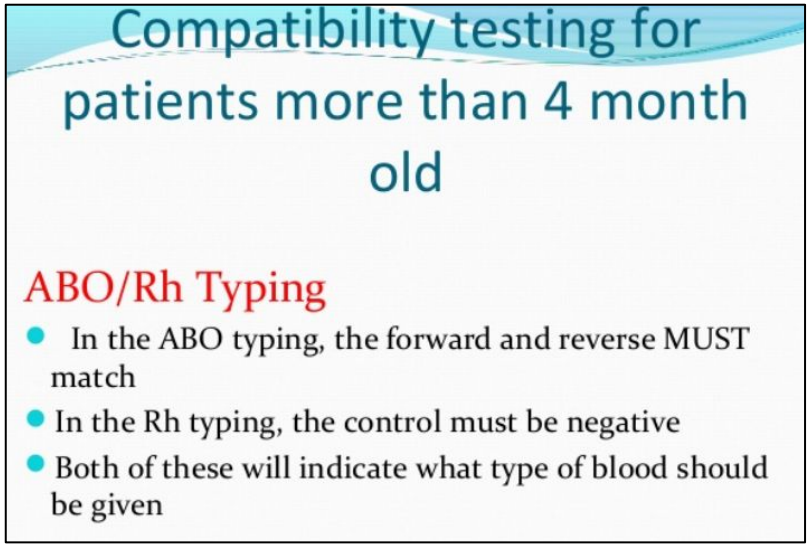
- If the sample is drawn from an IV line, the IV infusion should be stopped 5-10 minutes prior to blood drawing and the first 10 mL discarded
- Testing should be performed on samples **less than 72 hours** .

Getting the history

- Look at recipient's records for any prior unexpected antibodies
- Previous transfusion reactions

Serological Testing

- 3 tests:
  - ABO/Rh
  - Antibody detection/identification
  - Crossmatch



Rh typing

If your blood cells clump when mixed with anti-Rh antibodies, you have Rh+ blood. If they don't clump, you have Rh- blood.

ABO TYPING:

If your blood cells clump only when mixed with:

- anti-A antibodies, you have type A blood
- anti-B antibodies, you have type B blood
- both anti-A and anti-B antibodies, you have type AB blood

If your blood cells don't clump when mixed with either anti-A or anti-B antibodies, you have type O blood.

Back typing

If your serum causes clumping only when mixed with:

- type B cells, you have type A blood
- type A cells, you have type B blood
- type A and B cells, you have type O blood

If your serum doesn't cause clumping when mixed with either type A or B cells, you have type AB blood

Antibody screening

- Antibody screening is valid for 72 hr if it is negative and patient needs repeated transfusion , patient only need immediate spin crossmatch and ABO /Rh typing .
- Antibody screening valid for one week in patient have no history of transfusion or pregnancy in the last three months .

Antibody screen / Identification

- The antibody screen will detect the presence of any unexpected antibodies in patient serum
- If antibodies are detected, identification should be performed using panel cells (with an autocontrol)
  - IS
  - 37° (LISS)
  - AHG
- If an antibody is present, units negative for the antigen must be given after crossmaching.

Cross matching

- **Purpose**:
  - Prevent transfusion reactions
  - Increase *in vivo* survival of red cells
  - Double checks for ABO errors
  - Another method of detecting antibodies

Crossmatching

If your blood cells clump when mixed with a donor sample, the donor blood or organ is incompatible with your blood.

Selection of ABO Compatible Donor WB

WB= Whole Blood

Recipient	1st Choice
O Group	O
A Group	A Group
B Group	B Group
AB Group	AB Group

Selection of ABO Compatible Donor PRBCs

PRBC= Packed Red Blood Cells

Recipient	1st Choice	2nd Choice	3rd Choice	4th Choice
O Group	O	Non	Non	Non
A Group	A Group	O	Non	Non
B Group	B Group	O	Non	Non
AB Group	AB Group	A Group	B Group	O

Suggested ABO Group Selection Order for Plasma , plasma cryoprecipitate

Recipient	1st Choice	2nd Choice	3rd Choice	4th Choice
O	O	AB	A	B
A	A	AB		
B	B	AB		
AB	AB			

Suggested ABO Group Selection Order for Platelets

ABO	1st Choice	2nd Choice	3rd Choice	4th Choice
O	O	AB	A	B
A	A	AB	(B)	(O)
B	B	AB	(A)	(O)
AB	AB	(A)	(B)	(O)

Sample storage

	24c– 18	4c	18c–
EDTA whole blood	Up to 48 hr	Up to 7 days	-----
Serum	-----	Up to 7 days	Up to 6 months

Issuing blood

- When it's time to release a blood product to the nurse or physician, a few "checks" must be done
  - Requisition form
  - Comparing requisition form donor unit tag blood product label
  - Name of persons issuing and picking up blood
  - Date and time of release
  - Expiration date
  - Visual inspection for the units .

Post-analytical phase

- Involves labeling, inspecting, and issuing the blood unit
- Labeling form includes patient's full name, ID number, ABO/Rh of patient and unit, donor #, compatibility results, and tech ID
- Form is attached to the donor unit and only released for the recipient
- The unit is visually inspected for abnormalities, such as bacterial contamination, discoloration , haemolysis , leaking , clots .

Starting Transfusion

- Sign the "Blood Transfusion Report that is attached to the product and fill in the start time.
- Monitor the patient throughout the transfusion for signs of an adverse reaction.
- Monitor infusion rates. Slower infusion rates may be necessary in infants, the elderly or patients that are cardiovascularly compromised or at risk for fluid overload .
- Patient vital signs must be checked and documented . It is recommended that vital signs be monitored:-
  - Within one hour prior to starting the transfusion .
  - After the first 15 minutes of the transfusion .
  - Every hour during the transfusion .
  - One hour following completion of the transfusion .

- Immediately document on the patient record at the time of transfusion:
  - The unit number.
  - Type of blood component or blood product transfused.
  - Date and time of start and finish.
  - Identity of individual who administered the transfusion.
  - If the patient experienced an adverse transfusion reaction.

- If a product is still hanging at the end of four hours from dispensing, discontinue and discard the remaining product

Potential Major Adverse reaction of Blood Transfusion

- Acute and delayed haemolytic transfusion reactions
- Minor and major febrile reactions
- Minor and major allergic reactions
- Volume overload
- Transfusion related acute lung injury
- Viral infections (HIV, HBV, HCV, etc)

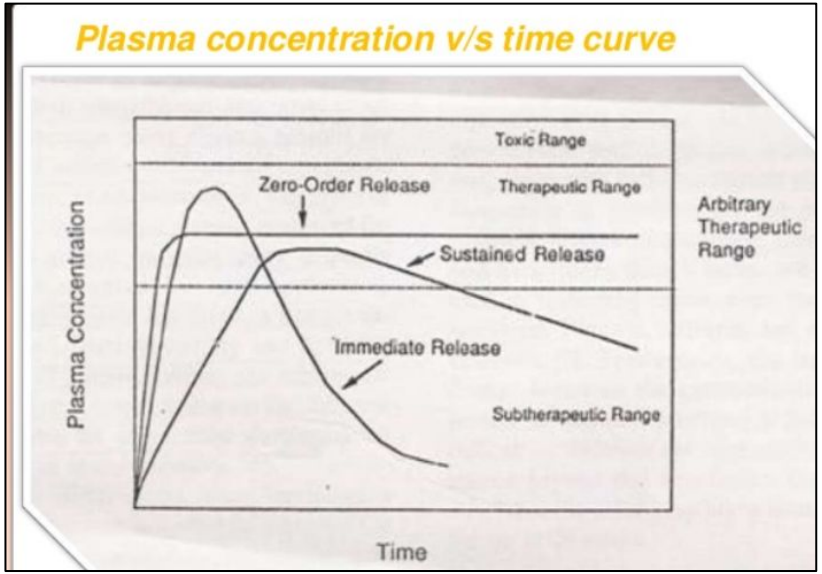
Advanced Sustained Release

DRUG DELIVERY SYSTEMS:

- The term "drug delivery systems" refer to the technology utilized to present the drug to the desired body site for drug release and absorption.
- Sustain Release Dosage Form:
  - Drug Delivery system that are designed to achieve prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose.
  - The basic goal of therapy is to achieve steady state blood level that is therapeutically effective and non toxic for an extended period of time.
  - The design of proper dosage regimen is an important element in accomplishing this goal.

- The **difference between controlled release and sustained release,**
  - **Controlled drug delivery**- which delivers the drug at a predetermined rate for a specified period of time
  - Controlled release is perfectly zero order release that is the drug release over time irrespective of concentration.
  - **Sustain release dosage form**- is defined as the type of dosage form in which a portion i.e. (initial dose) of the drug is released immediately, in order to achieve desired therapeutic response more promptly, and the remaining (maintenance dose) is then released slowly thereby achieving a therapeutic level which is prolonged,but not maintained constant.
  - Sustained release implies slow release of the drug over a time period.

Plasma concentration v/s time curve



Factors to be considered In S.R.Dosage forms.

1.Biological Factors

1. Absorption.
2. Distribution.
3. Metabolism.
4. Biological half life.(excreation)
5. Margin of safety

Physiological Factors:

1. Dosage size.
2. Partition coefficient and molecular size.
3. Aqueous Solubility.
4. Drug stability.
5. Protein binding.
6. Pka

Comparison between conventional and sustained-release drugs

Conventional Drug Therapy	Sustained-Release Drug Therapy
1. Rapid and complete release of drug immediately after administration.	1. Slow/controlled release of drug over an extended period of time.
2. Absorption is the rate-limiting step (kr >>> ka).	2. Drug release from the dosage form is the rate-limiting step (ka >>> kr).
3. Blood level fluctuates (Peak and Valley).	3. Constant blood level is maintained over a prolonged period (Reduced fluctuation).
4. There is risk of overmedication or under medication at periods of time.	4. Reliable therapy as the risk is minimized.
5. Frequent dosing.	5. Reduced frequency of dosing.
6. Patient non compliance. Therapeutic inefficiency / failure.	6. Improved patient compliance.
7. Inconvenience of patient.	7. Enhanced patient convenience with day-time and night-time medication.
8. No therapeutic action during overnight no dose period.	8. Maintains therapeutic action during overnight no dose period.
9. Risk of symptom breakthrough.	9. Improved treatment of many chronic diseases (minimizing symptom breakthrough).
10. Incidence and severity of untoward effects related to high -peak plasma concentration ↑.	10. Incidence and severity of untoward effects related to high – peak plasma concentration ↓.
11. More total dose over the entire course of therapy.	11. Less total dose over the entire course of therapy.
12. More side effects.	12. Minimize/eliminate incidence of local/systemic side effects.
13. Health care cost ↑.	13. Health care cost ↓.
14. Permits prompt testing of therapy.	14. Does not prompt.
15. Incidence of severity of GI side effects due to dose dumping of irritant drugs ↑.	15. Incidence of severity of GI side effects due to dose dumping of irritant drugs ↓.
16. More flexibility for physician in adjusting dosage required.	16. Less flexibility.

- **Sustained Release Drug Delivery System (SRDDS)** is designed to release a drug at a predetermined rate by maintaining a constant drug level for a specific period of time with minimum side effects.
- Now a day's focus on the development of SRDDS has increased, as very few drugs are coming out of research and development and already existing drugs are suffering the problem of resistance due to their irrational use specifically in case of drugs like antibiotics.
- SR dosage forms are designed to transport the blood level of a drug instantly to therapeutic concentrations by means of an initial dose portion and then maintain this level for a certain predetermined time with the continuation portion.
- SR of drugs in GI tract following oral administration is not affected by the absorption process.
- Drugs having a short half-life are eliminated quickly from blood circulation require frequent dosing. To avoid this problem oral sustained release formulations have been developed in an attempt to release the drug slowly into the g.i.t and maintain a constant drug concentration for long period of time.

Objectives of oral sustained released dosage form:

•To maintain the concentration of drug at constant level for a desired period of time.

•To reduce the frequency of doses administered as compared to conventional dosage form

•It should deliver active entity directly to site of action, minimizing or eliminating side effects.

•This may necessitate delivery to specific receptors or to localization to cells or to specific areas of the body.

•The safety margin of potent drugs can be increased.

•Incidence of both local and systemic adverse side effects can be reduced in sensitive patient

Challenges to sustained release drug delivery

1.Biocompatibility

2.Cost of formulation, preparation and processing

3.Fate of controlled release system if not biodegradable

4.Fate of polymer additives, e.g., plasticizers, stabilizers, antioxidants, fillers etc

Types of polymer

Since the structural and physicochemical characteristics of the polymer are decisive in the drug release mechanism,some will be more suitable than others, depending on the aim pursued and the drug desired

Hydrophilic polymers

a) Cellulosic

- Methylcellulose
- Hydroxypropylmethylcellulose (Hypromellose, HPMC)
- Hydroxypropylcellulose (HPC)
- Hydroxyethylcellulose (HEC)
- Ethylhydroxyethylcellulose (E-HEC)
- Sodium carboxymethylcellulose (Na-CMC)

b)Non-cellulosic

- Sodium alginate
- Xanthan gum
- Carrageenan
- Chitosan
- Guar gum
- Pectin
- Cross-linked high amylose starch
- Polyethylene oxide
- Homopolymers and copolymers of acrylic acid

Hydrophobic polymers

- Ethylcellulose
- Hypromellose acetate succinate
- Cellulose acetate
- Cellulose acetate propionate
- Methacrylic acid copolymers
- Polyvinyl acetate

1. Oral forms
2. Parenteral forms
3. Common sustained action dosage forms
    a. Spansules
    b. Slow core release tablets
    c. Multilayer tablets
    d. Repeat action tablets
    e. Liquid products
    f. Transdermal system

DESIGN OF ORAL SUSTAINED ACTION PRODUCTS

Formulation methods used to obtain the desired drug availability rate from sustained action dosage form include…….

- Increasing the particle size of the drug.
- Embedding the drug in matrix.
- Coating the drug or dosage form containing drug(microencapsulation).
- Forming complexes of the drug with material such as ion exchange resins.

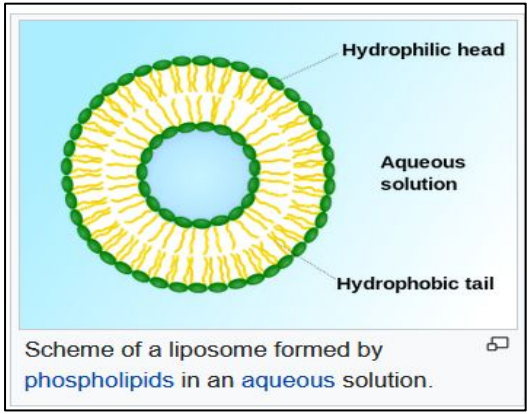
Since sustained release drug delivery system, provides increased bioavailability of drug product, reduction in the frequency of administration to prolong duration of effective blood levels, reduces the fluctuation of peak trough concentration and side effects and possibly improves the specific distribution of the drug, Sustained release formulations are a promising way to improve the patient compliance by reducing dosing interval and minimizing adverse effect.

Advanced drug Delivery Systems

- **Drug delivery r**efers to technologies, formulations based on drug vehicles or carriers, and approaches (routes of administration) used **to deliver drugs for various applications or therapeutic use.**
- **Advanced drug delivery systems (ADDS) r**efer to the technologies used for **controlling the rate of drug release.** The type of technology used greatly depends on the type of disease, type of drug and desired effect.
- Advanced formulations and use of novel drug vehicles have proven to increase the efficiency and efficacy of drug delivery systems, resulting in better treatment outcomes.
- Although the quest for advanced drug delivery systems and formulations began long ago, ADDS have gained more momentum in the past few decades with the development and advancement of formulations and technologies such as modified-release technologies, targeted-release technologies and formulations based on nanoparticles, microparticles, polymer conjugates, interfering RNA (siRNA), monoclonal antibodies, intelligent/smart polymers, osmotically modulated drug delivery, liposomes and dendrimers
- **Oncology is the largest application area in the advanced drug delivery marke**t due to the increasing use of new drug carriers and materials in targeted-release and controlled-release drug delivery, which has led to better drug formulations meeting unmet market needs.

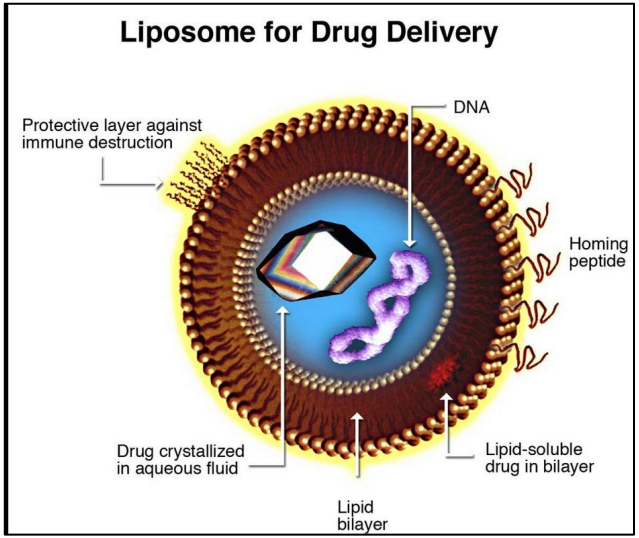
Liposome mediated drug delivery system:

- The word *liposome* derives from two Greek words: *lipo* ("fat") and *soma* ("body"); it is so named because its composition is primarily of phospholipid.
- A liposome is a spherical vesicle having at least one lipid bilayer and are formed by phospholipids (amphiphilic molecules having a hydrophilic head and hydrophobic tail).
- They form lamellar sheets when dispersed in aqueous medium by aligning themselves in such a way that the polar head group faces outwards the aqueous region while fatty acid groups face each other forming a spherical, vesicle like structures called as liposomes
- The **unique ability** of liposomal systems to entrap both lipophilic and hydrophilic compounds enables a diverse range of drugs to be encapsulated by these vesicles.
- Hydrophobic molecules are inserted into the bilayer membrane
- hydrophilic molecules can be entrapped in the aqueous center



Scheme of a liposome formed by phospholipids in an aqueous solution.

- The large aqueous center and biocompatible lipid exterior permits the delivery of a variety of macromolecules, such as DNA, proteins and imaging agents
- As a drug delivery system, liposomes offer several advantages including
    - biocompatibility,
    - capacity for self-assembly,
    - ability to carry large drug payloads,
    - a wide range of physicochemical and biophysical properties that can be modified to control their biological characteristics



Mechanism of action of liposomes:

Steps involved in liposome action of drug delivery:

1. Adsorption: Adsorption of liposomes to cell membranes causes its contact on the cell membrane.

2. Endocytosis: Adsorption of liposomes on the cell surface membrane followed by engulfment and internalization into the liposomes

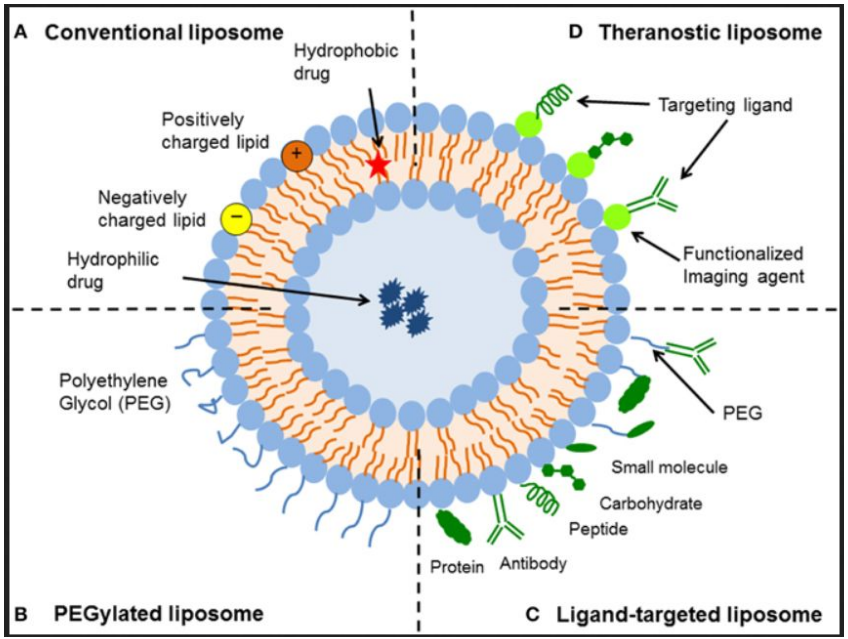
3. Fusion: fusion of lipid bilayers of liposomes with the lipoidal cell membrane by lateral diffusion and intermingling of lipids results in direct delivery of liposomal contents in the cytoplasm.

4. Lipid exchange: Due to the similarity of liposomal lipid membrane with cell membrane phospholipids, lipid transfer proteins in the cell membrane easily recognize liposomes and cause lipid exchange.

Classification Of Liposomes

- On the basis of size and number of bilayers:
  - multilamellar vesicles (MLV), large unilamellar vesicles (LUV) and small unilamellar vesicles (SUV).
- Based on composition:
  - conventional liposomes (CL), pH-sensitive liposomes, cationic liposomes, long circulating liposomes (LCL) and immuno-liposomes.
- Based on the method of preparation:
  - reverse phase evaporation vesicles (REV), French press vesicles (FPV) and ether injection vesicles (EIV)

S. No.	Classification based on structural features	Classification based on method of liposome preparation	Classification based on targeting concepts of liposomes
1	Multilamellar large vesicles	Single or oligolamellar vesicle made by reverse phase evaporation method.	PEGylated liposomes
2	Oligolamellar vesicles	Multilamellar vesicles made by reverse phase evaporation method.	Immunoliposomes
3	Unilamellar vesicles	Stable plurilamellar vesicles.	Cationic liposomes
4	Small unilamellar vesicles	Frozen and thawed MLV	Thermosensitive liposomes
5	Medium sized unilamellar vesicles	Vesicles prepared by extrusion method.	
6	Large unilamellar vesicles	Vesicles prepared by fusion	
7	Giant unilamellar vesicles	Vesicles prepared by French press	
8	Multivesicular vesicles	Dehydration-rehydration vesicles	
9		Bubblesomes	



Different types of liposomal drug delivery systems:

(A) Conventional liposome—Liposomes consist of a lipid bilayer that can be composed of cationic, anionic, or neutral (phospho)lipids and cholesterol, which encloses an aqueous core. Both the lipid bilayer and the aqueous space can incorporate hydrophobic or hydrophilic compounds, respectively.

(B) PEGylated liposome—Liposome characteristics and behavior *in vivo* can be modified by addition of a hydrophilic polymer coating, polyethylene glycol (PEG), to the liposome surface to confer steric stabilization.

(C) Ligand-targeted liposome—Liposomes can be used for specific targeting by attaching ligands (e.g., antibodies, peptides, and carbohydrates) to its surface or to the terminal end of the attached PEG chains.

(D) Theranostic liposome—A single system consist of a nanoparticle, a targeting element, an imaging component, and a therapeutic component.

Therapeutic Applications Of Liposomes

Liposomes provide superior therapeutic efficacy and safety in comparison to existing formulations. Some of the major therapeutic applications of liposomes in drug delivery include:

1. Site-avoidance delivery

The cytotoxicity of anti-cancer drugs to normal tissues is attributed to their narrow therapeutic index (TI). Under such circumstances, the TI can be improved by minimizing the delivery of drug to normal cells by encapsulating in liposomes.

For eg doxorubicin has a severe side effect of cardiac toxicity, but when formulated as liposomes, the toxicity was reduced without any change in the therapeutic activity

2. Intracellular drug delivery

Increased delivery of potential drugs to the cytosol (where drug receptors are present) can be accomplished by using LDDS. N-(phosphonacetyl)-L-aspartate (PALA) is normally poorly taken up into cells. Such drugs when encapsulated within liposomes, showed greater activity against ovarian tumor cell lines in comparison to free drug

3. Sustained release drug delivery

To achieve the optimum therapeutic efficacy, which requires a prolonged plasma concentration at therapeutic levels, liposomes provide sustained release of target drugs. Drugs like cytosine Arabinoside can be encapsulated in liposomes for sustained release and optimized drug release rate *in vivo*.

4. Intraperitoneal administration

Tumors that develop in the intra-peritoneal (ip) cavity can be treated by administering the drug to ip cavity. But the rapid clearance of the drugs from the ip cavity results in minimized amount of drug at the diseased site. However, liposomal encapsulated drugs have lower clearance rate, when compared to free drug and can provide a maximum fraction of drug in a prolonged manner to the target site

5. Immunological adjuvants in vaccines

Liposomes can be used for enhancing the immune response by encapsulating the adjuvants. Depending on the lipophilicity of antigens, the liposome can accommodate antigens in the aqueous cavity or incorporate within the bilayers. To enhance the immune response of diphtheria toxoid, liposomes were first used as immunological adjuvants

Nanoparticle mediated drug delivery systems:

- Nanotechnology-based pharmaceutics is a fast emerging field in the diagnosis and therapy of a number of human diseases, including cancer. Nanoparticles offer a stable means to achieve targeted drug delivery to various cells and tissues
- Various nanostructures including liposomes, polymers, dendrimers, and magnetic nanoparticles have been tested as carriers in drug delivery.
- Nanoparticles make it possible to achieve improved delivery of drugs which are poorly soluble in water by delivering drug of small particle size allowing faster dissolution in blood stream leading to targeted drug delivery in a cell- or tissue-specific manner.
- Particularly, the small size of nanoparticles facilitates their easy access to a wide range of cells and tissues.
- Further, the size of nanoparticles can be controlled and their surface can be modified with desired ligands and receptors to specifically target cells of interest as well as achieve controlled drug release
- **Due to their small size and large surface area**, drug nanoparticles show increase solubility and thus enhanced bioavailability, additional ability to cross the blood brain barrier (BBB), enter the pulmonary system and be absorbed through the tight junctions of endothelial cells of the skin
- Innovative drug delivery is driving the pharmaceutical companies to develop new formulations of existing drugs. While these new formulations will be beneficial to the patients, it will also create a powerful market force, driving the development of even more effective delivery methods

- “Nanotechnology” implies manipulation, reduction and fabrication of materials at nano scale with distinctive properties such as good strength, cost effective, lighter, eco friendly, definite and specific etc and enhanced functionality and improved stability

Properties of Nanoparticles that Made Them Suitable for Drug Delivery

- **Target specific**: used to deliver chemotherapeutics targeted to the tumor tissue without damaging normal organs
- **Chemically stable** structures that could be fabricated through different chemical and biological routes that are environment friendly and cost effective
- **Easily diffuse into the cell** by interacting with specific cellular components to permit selective targeting and accumulation in specific cell or tissues.
- **pH-labile structures** that easily degrades at low pH in the cell to release drug payloads. Examples of biodegradable nanoparticles polymeric polyethylene glycol
- **Persist for a longer period of time** without being degraded. Eg: carbon nanotube
- **Non-immunogenic** i.e., compatible with the body immune system. As a result will not engage in initiating an immune response when introduced in the body

Types of Nanoparticles for Drug Delivery:

Numerous different types of nanostructures exhibiting different physiochemical properties are employed to improve the efficiency of drug delivery to specific targets:

1. Polymerosomes:

- Synthetic amphiphilic blocks that are spherical vesicular bodies containing an aqueous solution.
- This unique copolymer is made of two components, a hydrophobic and hydrophilic subunits joined together.
- Eg: Poly lactic/glycolic acid, Polyethylene glycol-block-polycaprolactone

2. Nanocapsule:

- Are also known as liposomes which are a lecithin and stearate encapsulated structure with a lipid core where lecithin is sited in the inner part of the capsule.
- Polyethylene glycol could be employed to increase its half-life

3. Silica nanoparticles

- mesoporous nanoparticles with high specificity in terms of pore volume and surface area.
- used in imaging tools

4. Quantum dot:

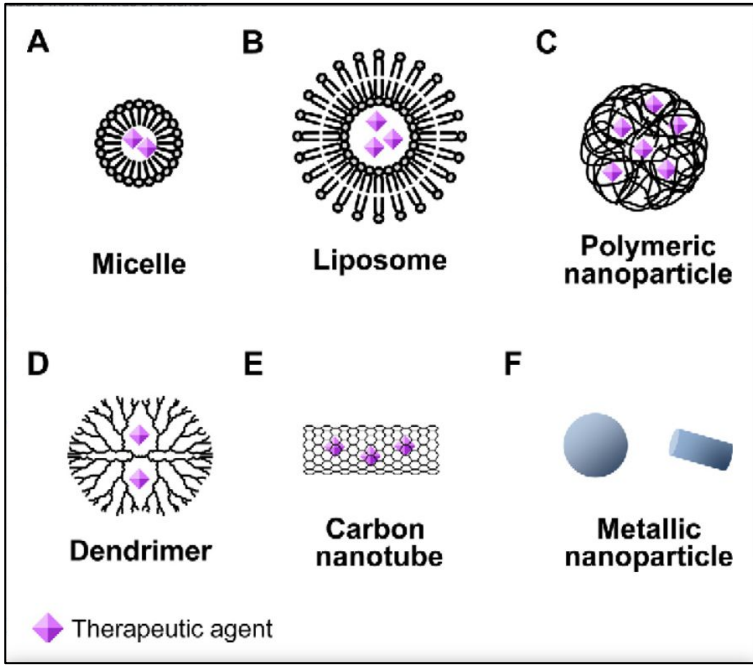
- Amalgamated nanoparticles composed of two subunits Poly L-lacticacid-block-polyethylene glycol.
- High optical resolution, small size and property of fluorochrome

5. Amphiphilic nanoparticles/ micelle

- dual nature nanoparticles consisted of hydrophobic and hydrophilic regions.
- Hydrophobic regions provide protection against the polar surroundings forming a micelle.
- In case of drug delivery it carries the drug and provides protection from the body's immune system to prevent its elimination

6. Dendrimers:

- Repeatedly branching nanostructures with highly specific large surface area making them target specific.
- It is mostly composed of polyamidoamine



7. Graphene:

- Allotropic form of carbon with unique thermochemical properties and high tensile strength.
- Its unique hexagonal structure enables infra-red radiation conversion in to heat.
- High surface area allows drug delivery in cancerous cell
- It is quite toxic in nature therefore requires surface shielding

8. Carbon nanotubes

- Basically graphene nanotubes used as hybrid drug carries.
- When bombarded with near-infrared radiation, it allows controlled drug release at specific tumor sites to protect the neighboring normal tissues

Drug Delivery Targeting

There are three main methods to transport drug-loaded nanoparticles to diseased sites

1. Passive targeting

- **Tumor cells have a property to absorb particles of specific size than normal cells**
- Nanoparticle morphology and electrochemical properties influence enhanced permeation and retention effect thus effecting drug penetration, duration of circulation and intracellular stability

2. Active targeting

- Active targeting involves the use of ligands such as antibodies,proteins, and peptides bound to nanoparticle surface to increase their uptake
- These ligands often protect nanoparticles from enzyme destruction.
- High binding affinity of ligands to the target cell will increase drug delivery efficiency

3. Physical targeting

- uses external sources or fields to guide the nanoparticle to the target site and also controls the release process

Therapeutic applications:

1.Cancer therapy

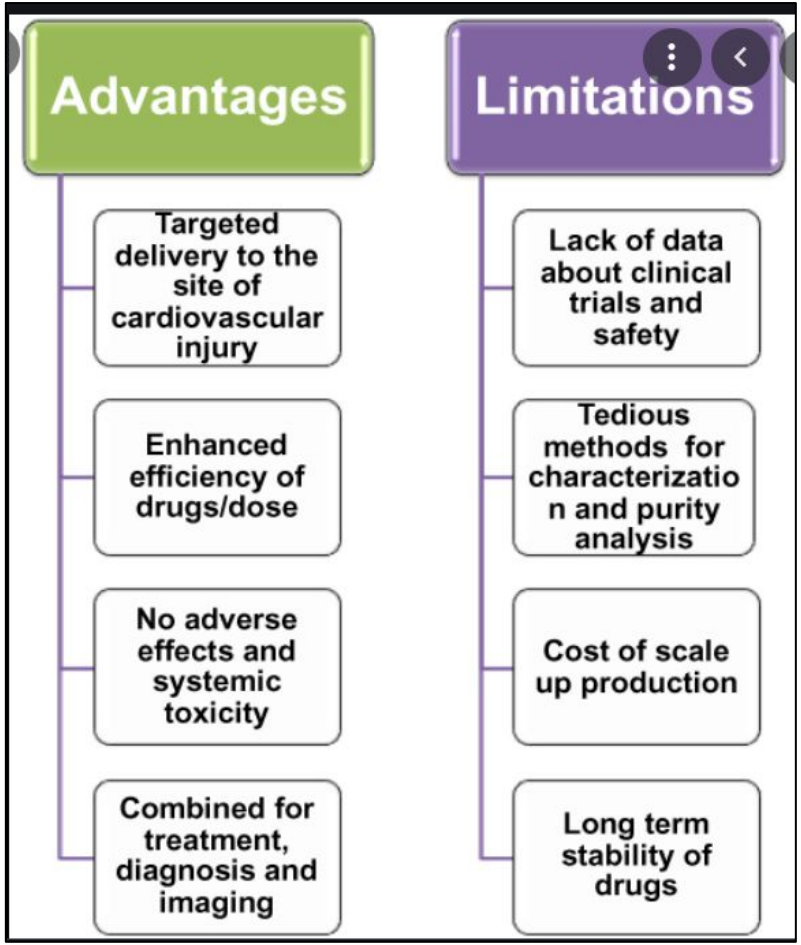
- With smartly designed nanoparticles, targeted drug delivery at the tumor site or a certain group of cells do largely avoid the toxic effects to other normal tissues and organs.
- **Micelles and liposomes** offer another option for delivery of chemotherapeutic agents. Additionally, **micelles** are also a great way to make insoluble drugs soluble due to their hydrophobic core and hydrophilic shell. If the **micelle's surface is further PEGylated**, it increases the ability of the nanocarriers to get through fenestrated vasculature of tumors and inflamed tissue through passive transport, thus resulting in higher drug concentration in tumors.
- A **polyfunctional dendrimer system** has been reported for successful localization (Folic acid), imaging (fluorescein) and delivery of the anticancer drug methotrexate in vitro
- Since **Carbon nanotubes** have very hydrophobic hollow interior, water insoluble drugs can easily be loaded them. The large surface area allows for outer surface functionalization and can be done specifically for a particular cancer receptor
- **Fullerene C60 c**an bind up to six electrons, and thus act as an excellent scavenger of reactive oxygen species (ROS)
- fullerene nanocrystals (Nano-C60) can enhance the cytotoxicity of chemotherapeutic agents

2. HIV and AIDS treatment

- Antiretroviral drugs must be able to cross the mucosal epithelial barrier when taken orally or other non-parental routes
- Lymphoid tissues are major sites for HIV to infect and thrive.
- nanoparticle loaded with antiretroviral drugs were able to target monocytes and macrophages in vitro
- poly(lactic-co-glycolic acid) (PLGA) was used to prepare nanoparticles entrapping three antiretroviral drugs, ritonavir, lopinavir, and efavirenz. The nanoparticle system yielded sustained drug release for over 4 weeks (28 days), while free drugs were eliminated within 48 h (2 days)
- The Central nervous system (CNS) is another site for HIV to inoculate and thrive resulting in serious HIV-associated neurocognitive disorder (HAND). Nanoparticles are a known to be able to cross BBB by endocytosis/phagocytosis and many reports exist showing successful delivery of anti-HIV medications

3.Nutraceutical delivery

- Most nutraceuticals are lipophilic molecules, such as fat-soluble vitamins (A, D, E and K), polyunsaturated lipids and other phytochemicals.
- A large number of nutraceuticals, posse anti-inflammatory, antioxidative, antiapoptotic, and antiangiogenic activities
- **curcumin** (diferuloylmethane) is practically water-insoluble and has very poor bioavailability, thus various methods have been implemented to address this issue, such as liposomes, phospholipid vesicles, and polymer-based nano-formulation
- A 9-fold higher oral bioavailability of curcumin was observed when compared to curcumin co-administered with piperine (absorption enhancer)
- **Resveratrol** is an important non-flavonoid polyphenol, naturally occurs in several plants. It is known for antioxidant, cardioprotective, anti-inflammatory and anticancer activities. has low solubility, with decent bioavailability, however, it is rapidly metabolized and eliminated
- polymeric nanoparticles, Zein based nanoparticles, nanoemulsions, liposomes , cyclodextrins and dual nanoencapsulation methods improve the pharmacokinetic profile and bioavailability


Advantages
Targeted delivery to the site of cardiovascular injury
Enhanced efficiency of drugs/dose
No adverse effects and systemic toxicity
Combined for treatment, diagnosis and imaging
Limitations
Lack of data about clinical trials and safety
Tedious methods for characterizatio n and purity analysis
Cost of scale up production
Long term stability of drugs

Biodegradable drug delivery system (hydrogel based)

- **Biodegradable Polymers:** are polymers that breakdown within a limited period of time after being placed in the body and are designed to offer temporary support.
- widely used as biomedical devices and in tissue engineering applications. Biodegradable polymers can either be natural or synthetic.
- The most widely used synthetic biodegradable polymers belong to the polyester family, such as Poly Lactic Acid (PLA) and Poly Glycolic Acid (PGA) and their copolymers, such as PGLA. have many applications including resorbable sutures , surgical fixation devices and drug delivery devices.
- Other commonly used biodegradable polymers include polydioxanone, which are primarily used as a suture material, marketed as Ethicon.
- The main advantage of biodegradable polymers is that their degradability reduces the need for subsequent surgical removal, saving time and money
- Many biodegradable polymers have been investigated for use in drug delivery and have established a role in controlled drug release. Eg: Aliphatic polyesters such as poly(lactic acid), poly(glycolic acid), poly(lactide-co-glycolide), poly(decalactone) and poly(ε-caprolactone)
- Many biodegradable polymers have been successfully fabricated into a number of devices for drug delivery including microspheres, microcapsules and nanoparticles

Hydrogels Based Drug Delivery:

- Hydrogels represent 3D polymeric networks specially designed for various medical applications.
- **Due to their porous structure,** they are able to swollen and to entrap large amounts of therapeutic agents and other molecules.
- In addition, their **biocompatibility and biodegradability properties**, together with **a controlled release profile,** make hydrogels a potential drug delivery system.

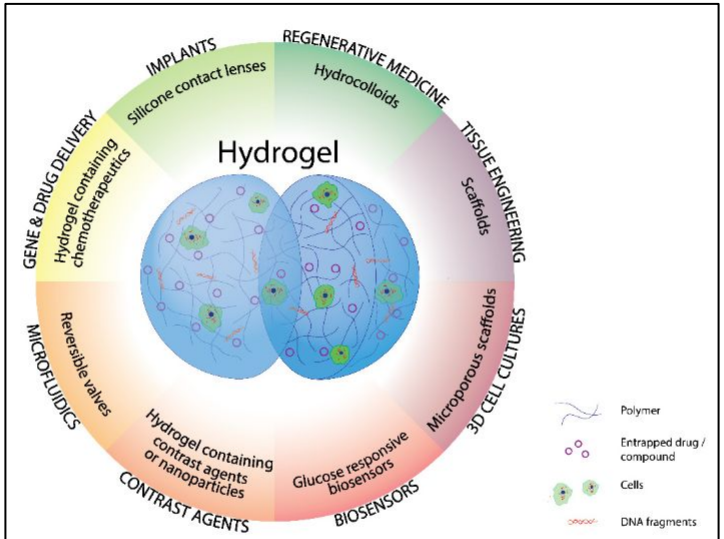


Figure 1. Hydrogel medical applications.

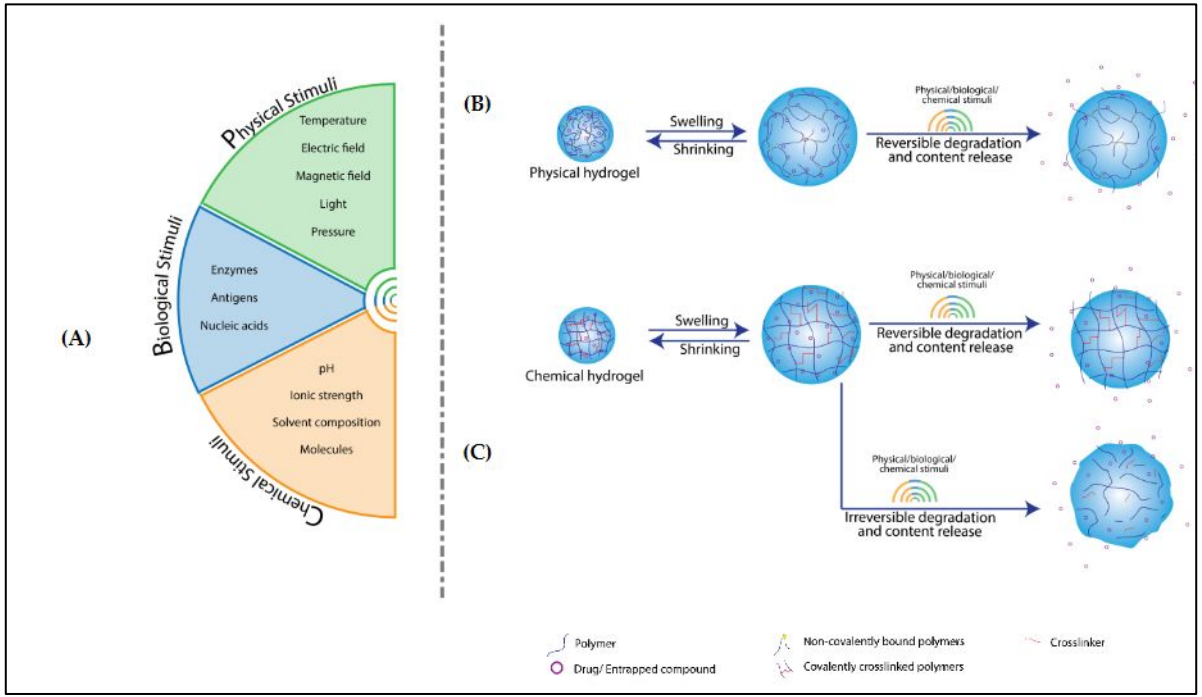


Figure 2. Stimuli sensitive hydrogels structural changes. **(A)** Stimuli categories: physical, biological and chemical. **(B)** Physical hydrogels are non-covalently crosslinked and in an aqueous environment, they swell. Under various stimuli presence, they undergo reversible structure modifications and release the compound. **(C)** Chemical hydrogels describes covalently crosslinked formulation that swells in aqueous conditions and then suffers reversible or irreversible alterations depending on stimuli presence and crosslinking strength, which influence their discharge.

Classification of hydrogels:

Hydrogels can be classified considering different parameters like: polymer origin, ionic chargeand biodegradability

Parameter	Hydrogel Type	Hydrogel Composition	Properties	Applications
Chemical stimuli	pH responsive	Carboxylated agarose/tannic acid hydrogel scaffolds cross-linked with zinc ions [60] Poly(acrylamide-*co*-acrylic acid) poly(AAm-*co*-AAc) hydrogels [61]	Sustained release of the incorporated drugs [60] Biocompatibility [60] Strong electrostatic interactions and stability [61] Increased hydrophilicity and swelling [62]	Drug delivery [54,60] Sensing [63]
	Ionic strength responsive	2-acrylamido-2-methylpropane sulfonic acid crosslinked with *N*,*N*′-methylene(bis)acrylamide [34] Poly(*N*-isopropylacrylamide) crosslinked with imidazolium-based dicationic ionic liquid [64]	Increased swelling properties [34] Controllable porous structure [34] Biodegradability [65]	Depollution of aqueous ecosystems [64] Drug and gene delivery [47] Tissue engineering [47]
	Solvent composition responsive	Fluorenylmethoxycarbonyl diphenylalanine [36] Poly(*N*-isopropylacrylamide) and poly(*N*,Ndimethylacrylamide) mixtures [66] Poly(*N*-isopropylacrylamide) [67,68]	Uniform networks [36] Swelling behaviour responsive to temperature too [66] High porosity, Heterogeneous structure [67]	Sensing [68]
	Molecules responsive	*N*-isopropylacrylamide crosslinked with *N*,*N*′-methylenebis(acrylamide) [69] Acrylamide crosslinked with polyethylene glycol [70]	Achieves molecular recognition, High affinity and specificity [71] Controlled assembly [72] Controlled release [43] Enzyme responsive [70] Antigen responsive [69]	Sensing [73] Drug delivery [37] Cell culture [72]

Parameter	Hydrogel Type	Hydrogel Composition	Properties	Applications
Physical stimuli	Temperature responsive	*N*-trimethyl chitosan chloride polymers crosslinked with poly(ethylene glycol) and glycerophosphate [74] Poly(*N*-vinylcaprolactam) grafted with poly(ethylene oxide) [75] Poly(*N*-isopropylacrylamide) and aminated alginate [76] Poly(*N*-vinylcaprolactam) [77] Methoxy poly(ethylene glycol)-poly(pyrrolidone-*co*-lactide) [78]	Two categories: low critical solution temperature [74] and upper critical solution temperature [75] Sol –gel transition at 37 °C [79] Easy functionalization with drug molecules [80] Unique physical properties similar to extracellular matrix [81] Controlled degradation [76]	Tissue engineering [76–78,82], Drug delivery [80,82]
	Electric field responsive	Polypyrrole polymeric nanoparticles loaded in poly lactic-*co*-glycolic acid and poly(ethyleneglycol) hydrogel [83]	Controlled release of the cargo [84] depending on the strength or the duration of applied electric field [83] Biocompatibility, Minimal invasiveness [83]	Drug delivery [84]
	Magnetic field responsive	Hemicellulose crosslinked with O-acetyl-galactoglucomannan [85] Gelatin hydrogels loaded with magnetic nanoparticles [86]	Successful absorption and controlled release of drugs [85] Some of them dispose of anisotropic properties [87]	Tissue engineering [86] Microfluidics, drug delivery, contrast agents [88]
Physical stimuli	Light responsive	Hydroxypropyl methylcellulose and Carbopol hydrogels containing diclofenac-sodium chitosan microspheres [89] Poly[2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-*N*-(2-(methacryloyloxy)ethyl)-*N*,*N*-dimethyl-2-oxoethan-1-aminium] [90]	Reasonable strengthens according to application [89] Reversible and irreversible, Spatiotemporal control over functional groups, Controlled release [91]	Drug delivery [89] Self-sterilization and self-cleaning [90] Microfluidics [92]
	Pressure responsive	Polyacrylamide and poly(acrylamide-hydroxyethyl methacrylate) [93]	Thermo- and pH sensitive [94] Adhesion capacity, elasticity [93]	Sensing [95] Drug delivery [96]

Parameter	Hydrogel Type	Hydrogel Composition	Properties	Applications
Polymer origin	Natural	Nanofibrillar cellulose [97] Thiolated gelatin-poly(ethylene glycol) diacrylate [98] Methacrylated gelatin [99] Polycaprolactone sandwiched in a gelatin-chitosan hydrogel [100]	Biomimetic and adhesion capacity [101] Mechanical support for cell development [102]	Tissue engineering [101,103,104], Drug delivery, Sensing [102]
	Synthetic	Low acyl gellan gum bilayered hydrogel scaffolds [105] *N*-isopropylacrylamide and itaconic acid [106] Poly(ethylene glycol)—poly(propylene glycol) copolymers [107]	Controllable structure and other physico-chemical properties [102] Stimuli responsive [106]	Drug delivery [106] Tissue engineering [108]
	Hybrid	Alginate-polymethacrylate [109] Chondroitin sulfate and poly(ethylene glycol) [110] Chitosan/hyaluronic acid hydrogels loaded with poly (lactic-*co*-glycolic acid) microspheres [111]	Biomimetic capacity [109] Multicomponent [112] Heterogeneous composition [113] Responsive to environment changes [114]	Tissue engineering, drug delivery [112] Wound-healing [111]
Biodegradability	Biodegradable	Chitosan-gelatin [115] Pectin-*co*-poly(methacrylic acid) [116]	Stable and biocompatible [116] Biomimetic capacity [117] Natural and synthetic polymeric structure [117] Stimuli responsive [118]	Drug delivery [116] Tissue engineering [117]
	Non-biodegradable	Poly(2-hydroxyethyl methacrylate) [58] Poly(2-hydroxyethyl methacrylate)/trimethylolpropane trimethacrylate [119]	Biocompatibility [58] Sustained release and recharge [119]	Tissue engineering [58] Drug delivery [119] Plastic and reconstruction surgery [120]

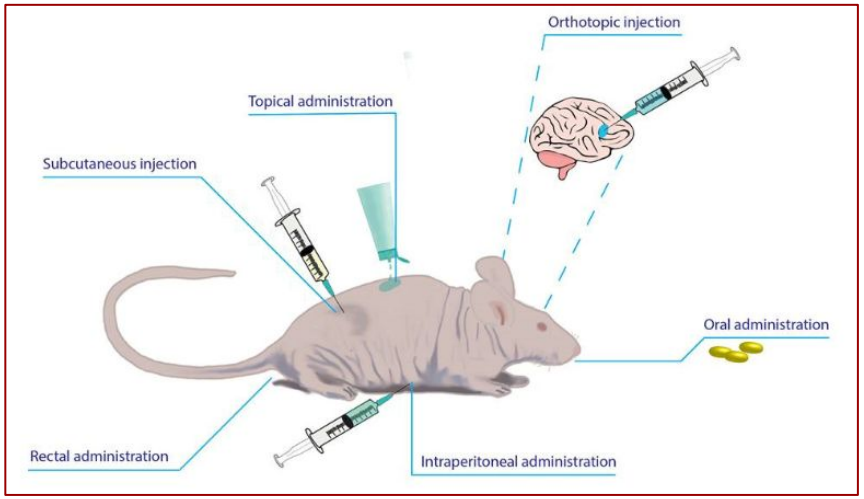
Hydrogel Functionalization with Therapeutic Agents:

- Crosslinking synthesis methods make possible the functionalization with drugs and other therapeutic agents in order to develop new delivery systems
- This procedure can be performed at two different times of hydrogel synthesis: at the beginning by mixing the drug with the other reagents or at the end after hydrogel is done
- **In situ loading method** suits for hydrophilic drugs and is based on dissolving the drug into the water together with the polymer powder.
- **Post-loading method** refers to dry hydrogel films immersion into drug solution for a certain period of time.
- In both of these cases, after drug incorporation, the hydrogel has a dried state and confers protection.
- In addition, **cross-linkers** are essential factors in **controlled release of high or low molecular weight therapeutic agents**, and in most cases the degradable cross-linkers are preferred

In Vivo Biocompatibility and Biodegradability:

- In order to compare and investigate the effects of hydrogels in vivo, it is necessary to evaluate its biocompatibility, since living organisms are prone to develop inflammatory reactions which are facilitated by the degradation of the synthetic polymers

- The over 100 years of research have produced gel-like biopolymers with low toxicity and high biocompatibility, especially those derived from natural molecules such as collagen, chitosan, fibrin, and hyaluronic acid
- The **most accessible routes of administration** are subcutaneous, oral, rectal, topical and transdermal, orthotopic, intraperitoneal and ocular



- Subcutaneous injection plays a crucial role in toxicological effects evaluation.
- Topical or transdermal application is preferred for skin associated problems.
- Orthotopic and intraperitoneal injections are non-invasive techniques which ensure good therapy results.
- On the other hand, oral administration has some limitations because of digestive enzymes

Clinical Trials:

- In the last decade, an increasing number of clinical trials investigated the use of hydrogelstructures for drug delivery systems.

Table 2. Different completed clinical trials using hydrogel based products (ClinicalTria

Condition	Product	Benefits
Prostate cancer radiotherapy	Hydrogel spacer	Minimal side effects and toxicity Improves rectal dosimetry Reduces the rates of rectal toxicity
Gynecologic laparoscopic surgery	Crosslinked hyaluronan gel	Safety use Minimizes postoperative adhesion formation throughout the abdominopelvic cavity
Corneal epithelial permeability	Silicone hydrogel contact lenses	Improves epithelial permeability when used with ophthalmic solutions
Corneal infiltrates		Identification of bacterial species during continuous wear of contact lenses Improved cornea response to contact lenses
Myopia		Good ocular comfort High oxygen transmissibility
Dry eye syndrome	Crosslinked hyaluronic acid with liposomes and crocin	Safety profile Promotes re-epithelialization
Urinary incontinence	Polyacrylamide hydrogel	Facilitates urinary incontinence symptoms for patients that are ineligible for midurethral sling surgery Low rate of adverse effects
Cerebrospinal fluid leak	Fibrin sealant	Efficient adjunct to dural sutures repair Safe profile
Ischemic cardiomyopathy	Gelatin hydrogel	Controlled release of fibroblast growth factor Increases the formation of cardiovascular networks Improves ventricular function
Heart failure	Alginate hydrogel	Efficiency and safety profile No serious adverse effects Increases exercise capacity
Elective cranial procedures with dural incision	PEG hydrogel	Safe profile Dural closure augmentation Rapid preparation and application
Neuropathic pain	Lidocaine plaster	Safety and tolerability profile Relevant pain reduction

Types and identification of stem cells

- In multicellular organisms, **stem cells** are undifferentiated or partially differentiated cells that can differentiate into various types of cells and proliferate indefinitely to produce more of the same stem cell.
- They are the earliest type of cell in a cell lineage
- .They are found in both embryonic and adult organisms, but they have slightly different properties in each.
- They are usually distinguished from progenitor cells, which cannot divide indefinitely, and precursor or blast cells, which are usually committed to differentiating into one cell type.

Properties:

- **Self-renewal:** the ability to go through numerous cycles of cell growth and cell division, known as cell proliferation, while maintaining the undifferentiated state.
- **Potency:** the capacity to differentiate into specialized cell types. Potency specifies the differentiation potential of the stem cell.
  - **Totipotent (also known as omnipotent) stem cells** can differentiate into embryonic and extraembryonic cell types. Such cells can construct a complete, viable organism
  - **Pluripotent stem cells** are the descendants of totipotent cells and can differentiate into nearly all cells, i.e. cells derived from any of the three germ layers
  - **Multipotent stem cells** can differentiate into a number of cell types, but only those of a closely related family of cells
  - **Oligopotent stem cells** can differentiate into only a few cell types, such as lymphoid or myeloid stem cells
  - **Unipotent cells** can produce only one cell type, their own but have the property of self-renewal, which distinguishes them from non-stem cells (e.g. progenitor cells, which cannot self-renew).

Classification of stem cells (SCs)

Classification		Characteristics
Sources/types	Embryonic stem cells	are pluripotent stem cells derived from the inner cell mass of the blastocyst, an early-stage embryo.
	Adult stem cells	Endodermal Origin: Pulmonary Epithelial SCs, Gastrointestinal Tract SCs, Pancreatic SCs, Hepatic Oval Cells, Mammary and Prostatic Gland SCs, Ovarian and Testicular SCs
		Mesodermal Origin: Hematopoietic SCs, Mesenchymal Stroma SCs, Mesenchymal SCs, mesenchymal precursor SCs, multipotent adult progenitor cells, bone marrow SCs, Fetal somatic SCs, Unrestricted Somatic SCs, Cardiac SCs, Satellite cells of muscle
		Ectodermal Origin : Neural SCs ， Skin SCs ， Ocular SCs
	Cancer stem cells	have been identified in almost all caner/tumor, such as Acute Myeloid leukemic SCs ($CD34^+/CD38^{-}$), Brain tumor SCs ($CD133^+$), Breast cancer SCs ($CD44^+/CD24^-$), Multiple Myeloma SCs ($CD138^+$), Colon cancer SCs ($CD133^+$), Liver cancer SCs ($CD133^+$), Pancreatic cancer SCs ($CD44^+/CD24^+$), Lung cancer SCs ($CD133^+$), Ovary cancer SCs ($CD44^+/CD117^+$), Prostate cancer SCs ($CD133^+/CD44^+$), Melanoma SCs ($CD4^+/CD25^+/FoxP3^+$), Gastric cancer SCs ($CD44^+$).
	Induced pluripotent stem cells	a type of pluripotent stem s artificially derived from a non-pluripotent cell, typically an adult somatic cell, by inducing a "forced" expression of specific genes.

Cell potency	Totipotent cells	Zygote, Spore, Morula; It has the potential to give rise to any and all human cells, such as brain, liver, blood or heart cells. It can even give rise to an entire functional organism.
	Pluripotent cells	Embryonic stem cell, Callus; They can give rise to all tissue types, but cannot give rise to an entire organism.
	Multipotent cells	Progenitor cell, such as hematopoietic stem cell and mesenchymal stem cell; They give rise to a limited range of cells within a tissue type.
	Unipotent cells	Precursor cell

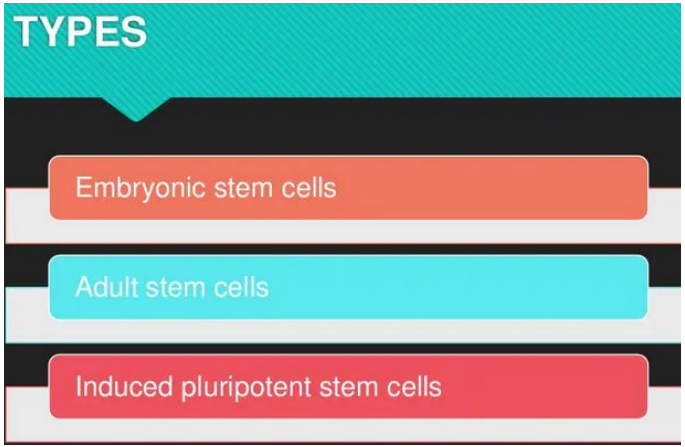
Identification of stem cells: In practice, stem cells are identified by whether they can regenerate tissue.

- **Stem cell cultures**

Stem cells are either extracted from adult tissue or from a dividing zygote in a culture dish and then allowed to divide and replicate.

- **Stem cell lines**

Once stem cells have been allowed to divide and propagate in a controlled culture, the collection of healthy, dividing, and undifferentiated cells is called a stem cell line. These stem cell lines are subsequently managed and shared among researchers.



EMBRYONIC STEM CELLS

OBTAIN

- These are derived from embryos which are fertilized in vitro

GROWTH

- generated by transferring cells from a preimplantation-stage embryo into a plastic laboratory culture dish that contains a nutrient broth known as culture medium
- the inner surface of the culture dish was coated with mouse embryonic skin cells called a feeder layer.
- The mouse cells in the bottom of the culture dish provide the cells a sticky surface to which they can attach. Also, the feeder cells release nutrients into the culture medium.

DIFFERENTIATION

Embryonic stem cells that have proliferated in cell culture for six or more months without differentiating, are pluripotent, and appear genetically normal are referred to as an embryonic stem cell line

IDENTIFICATION

This process is called characterization
Growing and subculturing the stem cells for many months to see that the cells look healthy and remain undifferentiated.
Transcription factors help turn genes on and off at the right time, which is an important part of the processes of cell undifferentiation and embryonic development e.g Nanong & Oct 4

Testing whether the human embryonic stem cells are pluripotent by

1. allowing the cells to differentiate spontaneously in cell culture
2. manipulating the cells so they will differentiate to form cells characteristic of the three germ layers
3. injecting the cells into a mouse with a suppressed immune system to test for the formation of a benign tumor called a teratoma

Advantages

- Make all the different types of cells in our body.
- Can self-renew forever so large supplies can be made.

ADULT STEM CELLS

Also called somatic stem cells

OBTAIN

- Found among differentiated cells in a tissue including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis

GROWTH

- Once removed from the body, their capacity to divide is limited, making generation of large quantities of stem cells difficult.

DIFFERENTIATION

Hematopoietic stem cells give rise to all the types of blood cells: red blood cells, lymphocytes, natural killer cells,monocytes, and macrophages.
Mesenchymal stem cells have been reported to be present in many tissues. Those from bone marrow (bone marrow stromal stem cells, skeletal stem cells) give rise to a variety of cell types
Neural stem cells in the brain give rise to its three major cell types: nerve cells and non-neuronal cells—astrocytes and oligodendrocytes.
Epithelial stem cells in the lining of the digestive tract give rise to several cell types: absorptive cells, goblet cells, and enteroendocrine cells.
The epidermal stem cells give rise to keratinocytes
The follicular stem cells can give rise to both the hair follicle and to the epidermis.

IDENTIFICATION

1. label the cells in a living tissue with molecular markers and then determine the specialized cell types they generate
2. remove the cells from a living animal, label them in cell culture, and transplant them back into another animal to determine whether the cells replace their tissue of origin.

ADVANTAGE

- Make only the types of cells that belong in their own tissue, e.g skin stem cells make only types of skin cells ,they do not make brain cells
- Already partly specialised, which can make it more straightforward to obtain the particular specialised cell type required

INDUCED PLURIPOTENT STEM CELLS

OBTAIN

- derived from adult cells in 2007 - very recent discovery!

GROWTH

- can be grown indefinitely in culture in an undifferentiated state

DIFFERENTIATION

- similar properties to embryonic stem cells as can differentiate into many different tissue types – **pluripotent**

ADVANTAGE

- can create stem cells directly from a patient for research

Signals to Stem Cells

- Cells respond to molecules in their extracellular environment. They also respond to chemicals or molecules floating around in the liquid surrounding them.
- Cells can feel and communicate with each other, and an embryonic stem cell may respond very differently to contact with a bunch of muscle cells than to a bunch of neurons.
- Stem cell can make a decision to self-renew or differentiate based on the individual components.

Embryonic stem cells in the dish: How do we culture ES cells?

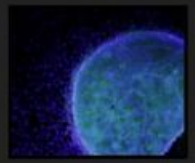
- There are THOUSANDS of HUMAN embryonic stem cells growing in this circular colony. The HUMAN embryonic stem cells are grown on top of MOUSE fibroblast cells—the elongated cells—which provide essential nutrients.
- When we culture embryonic stem cells, we are trying to stop the majority of the colony from differentiating so that we can maintain a pure population of proliferating embryonic stem cells. This is so we have a continuing supply for research purposes.

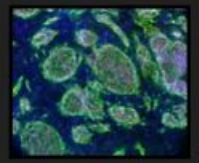
Culture methods

- Cells are cultured in plastic petri dishes or plates.
- Everything is kept sterile, or closed off to the air, to prevent contamination by bacteria, fungi, and viruses.
- First, you coat a plate with a thin layer of gelatin, and let it set. Then, you pipette Mouse Embryonic Feeder cells onto the gelatin, and let them grow. Next, you thaw a tube of human embryonic stem cells, and take the tube of cells underneath a fume hood. The air in here is constantly filtered so it's for the most part sterile.
- Then TO the plate you add a specific culture media, the liquid solution that the cells need and take nutrients.
- To make this media, you add a culture solution at the correct pH for cells that also contains a color-changing pH indicator. Then you add a serum replacer.
- Next you add essential and non-essential amino acids AND a growth factor that promotes proliferation.
- Last, you drip the embryonic stem cells onto the plate with media, and put it into an incubator.
- The incubator is kept at around body temperature and with 5% Carbon dioxide in th air, same as in your body. This helps them grow and keep the correct ph.
- When you check up on the cells the next day, the color-changing pH indicator will t you the approximate pH of the cell media. If it is yellow to light orange, it has a lowe pH (or is slightly acidic). If it is red, then it has a higher pH (or is slightly basic).
- Human embryonic stem cells like to grow in a yellow to light orange media since they thrive in the lower ph.

Fluorescent imaging of embryonic stem cell colonies.

- Scientists can label embryonic stem cells using antibodies attached to fluorescent molecules.
- The smaller blue dots in the green area label the nuclei of the embryonic stem cells, and the blue dots outside the colony label fibroblast feeder cells. This is how scientists can keep track of which cells are stem cells and which are not.
- Embryonic stem cells like to grow in little colonies spaced out over the dish instead of in one big layer.





Applications

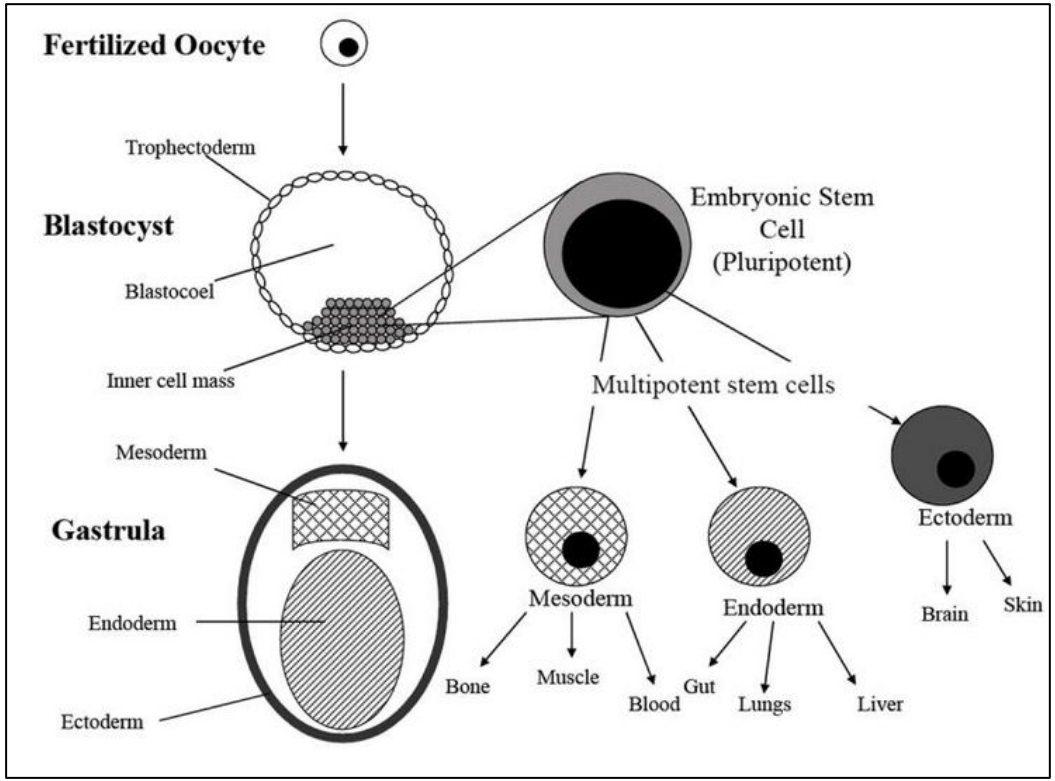
- Tissue repair

-nerve.heart,muscle,organ,skin

- Cancer
- Autoimmune diseases

-diabeties,rhematoid arthiritis,MS

Fate Mapping of Stem Cells



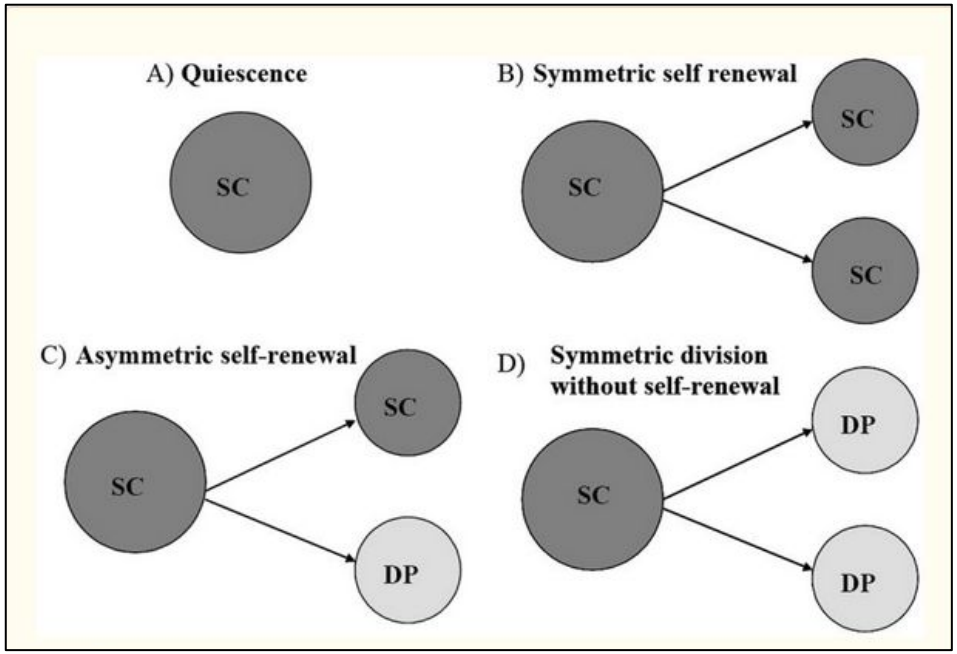
Derivation of Stem Cells

- During natural embryo development, cells undergo proliferation and specialization from the fertilized egg, to the blastocyst, to the gastrula during natural embryo development (left side of panel).
- Pluripotent, embryonic stem cells are derived from the inner cell mass of the blastoctyst (lightly shaded).
- Multipotent stem cells (diamond pattern, diagonal lines, and darker shade) are found in the developing gastrula or derived from pluripotent stem cells and are restricted to give rise to only cells of their respective germ layer.

Stem Cell Fates

Based on the two defining characteristics of stem cells (unlimited self-renewal and ability to differentiate), they can be described as having four outcomes or fates

- A **common fate** for multipotent stem cells is to **remain quiescent** without dividing or differentiating, thus maintaining its place in the stem cell pool. An example of this is stem cells in the bone marrow that await activating signals from the body.
- A **second fate** of stem cells is **symmetric self-renewal** in which two daughter stem cells, exactly like the parent cell, arise from cell division. This does not result in differentiated progeny but does increase the pool of stem cells from which specialized cells can develop in subsequent divisions.
- The **third fate**, **asymmetric self-renewal**, occurs when a stem cell divides into two daughter cells, one a copy of the parent, the other a more specialized cell, named a somatic or progenitor cell. Asymmetric self-renewal results in the generation of differentiated progeny needed for natural tissue development/regeneration while also maintaining the stem cell pool for the future.
- The **fourth fate** is that in which a stem cell divides to produce two daughters both different from the parent cell. This results in greater proliferation of differentiated progeny with a net loss in the stem cell pool.



Stem Cell Fates

Four potential outcomes of stem cells.

A) Quiescence in which a stem cell does not divide but maintains the stem cell pool.

B) Symmetric self-renewal where a stem cell divides into two daughter stem cells increasing the stem cell pool.

C) Asymmetric self-renewal in which a stem cell divides into one differentiated daughter cell and one stem cell, maintaining the stem cell pool.

D) Symmetric division without self-renewal where there is a loss in the stem cell pool but results in two differentiated daughter cells. (SC-Stem cell, DP-Differentiated progeny)

Fate mapping

- is a method used in developmental biology to **study the embryonic origin** of various adult tissues and structures.
- The "fate" of each cell or group of cells is mapped onto the embryo, showing which parts of the embryo will develop into which tissue.
- When carried out at single-cell resolution, this process is called cell lineage tracing.
- It is also used to trace the development of tumors

Method

- Fate mapping is accomplished by **inserting a heritable genetic mark** into a cell. Typically, this is a fluorescent protein.
- Therefore, any progeny of the cell will have this genetic mark.
- It can also be done through the **use of molecular barcodes,** which are introduced to the cell by retroviruses

Fate mapping is a technique used to understand how embryonic cells divide, differentiate, and migrate during development.

In classic fate mapping experiments, cells in different areas of an embryo are labeled with a chemical dye and then tracked to determine which tissues or structures they form.

Technological improvements now allow for individual cells to be marked and traced throughout embryonic development and adulthood.

Use of stem cells in therapy

- ***Stem cells* or mother or queen of all cells** are pleuropotent and *have* the remarkable potential to develop into many different cell types in the body.
- Serving as a sort of repair system for the body, they can theoretically divide without limit to replenish other cells as long as the person or animal is alive.
- When a stem cell divides, each new cell has the potential to either remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell.

- Stem cells may be the person's own cells (a procedure called ***autologous transplantation)*** or those of a donor (a procedure called ***allogenic transplantation***).
- When the person's own stem cells are used, they are collected before chemotherapy or radiation therapy because these treatments can damage stem cells. They are injected back into the body after the treatment.
- The **sources of stem cells** are varied such as pre-implantation embryos, children, adults, aborted fetuses, embryos, umbilical cord, menstrual blood, amniotic fluid and placenta
- New research shows that transplanted stem cells migrate to the damaged areas and assume the function of neurons, holding out the promise of therapies for *Alzheimer's disease, Parkinson's, spinal cord injury, stroke, Cerebral palsy, Battens disease and other neurodegenerative diseases.*

1. **Neurological disorder:**

- Neurological disease encompasses a diverse group of disorders of the central and peripheral nervous systems
- Specifically, aging can have detrimental effects on the progression of brain diseases and endogenous stem cells. Stem cell therapies possess promising potential to mitigate the neurological symptoms of such disease
- The scope of treatment options for neurological disease is limited, and drug approval rates for improved treatments remain poor when compared with other therapeutic areas. Stem cell therapy provides hope for many patients, but should be tempered with the realisation that the scientific and medical communities are still to fully unravel the complexities of stem cell biology
- Over 200 clinical studies applying various stem cell approaches to treat neurological disease have been registered to date , the majority of which are for multiple sclerosis, stroke and spinal cord injuries

Essential properties of stem cells for use in clinical transplantation

- Capable of clonal propagation in vitro to ensure homogeneity
- Genetic stability at high passage
- Integration within the host brain following transplantation
- Connectivity within host circuits
- Migration and engraftment at sites of damage
- Correct differentiation into appropriate neural cell types
- Functional benefits
- Lack of side effects

GOAL OF STEM CELL THERAPY

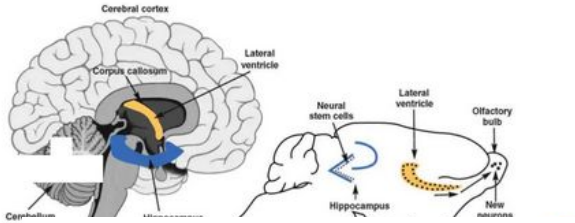
- Different body tissues/organs have different regenerative potentials after injury.
- To promote cell replacement in organs that are damaged beyond their ability to self-repair.

NEUROLOGICAL DISEASES

- **Trauma**-SCI
- **Vascular**-Stroke
- **Degenerative**- Parkinson's disease, Huntington's chorea, Amyotrophic lateral sclerosis, AD etc.
- **Chronic inflammatory and immune mediated-** multiple sclerosis
- **Genetic diseases in children-** neuronal ceroid lipofuscinosis, mucopolysaccharidoses, Leucodystrophies and muscular dystrophies.

SITES OF ADULT NEURONAL STEM CELLS

Subventricular zone(SVZ) of lateral ventricles and the Subgranular zone of dentate gyrus in hippocampus.



SOURCES OF STEM CELLS

- **Human embryonic stem cells (ESC)**
- **Human umbilical cord blood cells (UCB)**
- **Immortalised cell lines**
- **Foetal neural stem cells**
- **Adult neural stem cells (NSC)**
- **Bone marrow derived cells**
- **Induced pluripotent cells (iPS)**

HUMAN EMBRYONIC STEM CELLS (ESCS)

- Derived from the inner cell mass of the embryonic blastula(Pleuripotent)
- Can provide an unlimited source of cells
- Can be directed into neural precursors to generate neurons, oligodendroglia and astrocyte both *in vitro* and *in-vivo*.

HUMAN UMBILICAL CORD BLOOD CELLS (UCB)

- Derived from umbilical cord blood
- Can differentiate into neural lineages
- Produced progeny that shows positivity of neural and glial cells markers
- Experimental data in animal models of stroke have shown functional benefits
- A better understanding of these cells is needed before clinical transplantation studies ensue

IMMORTALISED CELL LINES

- As there is Ethical difficulties in transplanting embryonic cells .
- Derived by infecting neuroepithelial precursor cells from predefined CNS regions before terminal mitosis, with a retrovirus encoding an immortalizing oncogene.

FOETAL NEURAL STEM CELLS

- Harvested from the post-mortem human fetal brain
- Maintain a normal karyotype for a significant number of passages in culture .
- Can produce a large number of neurons and astrocytes.
- High proliferative capacity without any evidence of tumorogenesis .

ADULT NEURAL STEM CELLS (NSC)

- Multipotent stem cells found in developed organisms
- Identified within bone marrow, brain, heart, skin and bone.
- 1-2% of the total cell population within a particular tissue type.

ADULT NEURAL STEM CELLS (NSC)…

- Usually quiescent and in an undifferentiated state .
- Their proliferative capacity is limited.
- Can generate neurons, astrocytes and oligodendrocytes,

BONE MARROW DERIVED CELLS

- Mobilized Peripheral Blood (MPB) and MSCs are clinical source of HSCs.
- MPB contains a mixture of hematopoietic stem and progenitor cells
- These cells have the potential to regenerate the brain tissue by release of neurotrophic growth hormones

INDUCED PLURIPOTENT CELLS (IPS)

- Similar to human embryonic stem cells
- Adult human cells from skin were transformed to a pluripotent state using genetic engineering techniques
- Can differentiate into cell types of all the three germ layers.

SOURCES OF SCS

- **Autologous-**
  Source- peripheral blood, cord blood, bone marrow , skin
  Benefit- no risk of rejection
- **Allogeneic-**
  Source- HLA matched relative or unrelated donor
  Limitations- Histocompatibility
  Fetal tissue is also used, raising ethical issues.
- **Xenogenic-**
  Source- porcine fetal ventral mesencephalic (FVM) cells
  Limitations- Life long immunosupression and risk of rejection.

NUCLEAR REPROGRAMMING

- N. Development -totipotent (zygote) – pluripotent cells(blastocyst) - multipotent fertilized cells - terminally differentiated cells.
- The reversal of the terminally differentiated cells to totipotent or pluripotent cells (called **nuclear reprogramming)**.
- Achieved by using *nuclear transplantation, or nuclear transfer (NT), procedures*("cloning"), where the nucleus of a differentiated cell is transferred into an enucleated oocyte.

iPS-

- An alternative approach that has become a method of choice is induced pluripotent stem [iPS] by using various transcription factors(TFs)

Direct reprogramming-

- To convert one type of terminally differentiated cell (e.G., Fibroblast cell) into another type of terminally differentiated cell (e.G., Cardiac muscle, neuron, or hepatocyte) by overexpressing specific sets of TFs .
- This technology is currently limited by its low efficiency

- Lineage commited multipotent stem cells can show **transdifferentiation.** e.g., HS cells may be converted into neurons as well as germ cells.
- Tissue stem cells can be derived directly from a patient for therapeutic purposes.

The following diseases have been treated by various stem cell practitioners with generally positive results and the spectrum has ever since been increasing.

1. **Cerebral Palsy**

- Cerebral palsy is a disorder caused by damage to the brain during pregnancy, delivery or shortly after birth. It is often accompanied by seizures, hearing loss, difficulty speaking, blindness, lack of co-ordination and/or mental retardation
- It produces chronic motor disability in children. The causes are quite varied and range from abnormalities of brain development to birth-related injuries to postnatal brain injuries

2. **Alzheimer's Disease**

- Alzheimer's is a complex, fatal disease involving progressive cell degeneration, beginning with the loss of brain cells that control thought, memory and language.
- The disease, which currently has no cure, was first described by German physician Dr. Alzheimer
- *Neural stem cell (NSC)* grafts present a potential and innovative strategy for the treatment of many disorders of the central nervous system including AD, with the possibility of providing a more permanent remedy than present drug treatments. After grafting, these cells have the capacity to migrate to lesioned regions of the brain and differentiate into the necessary type of cells that are lacking in the diseased brain, supplying it with the cell population needed to promote recovery

3. **Multiple Sclerosis**

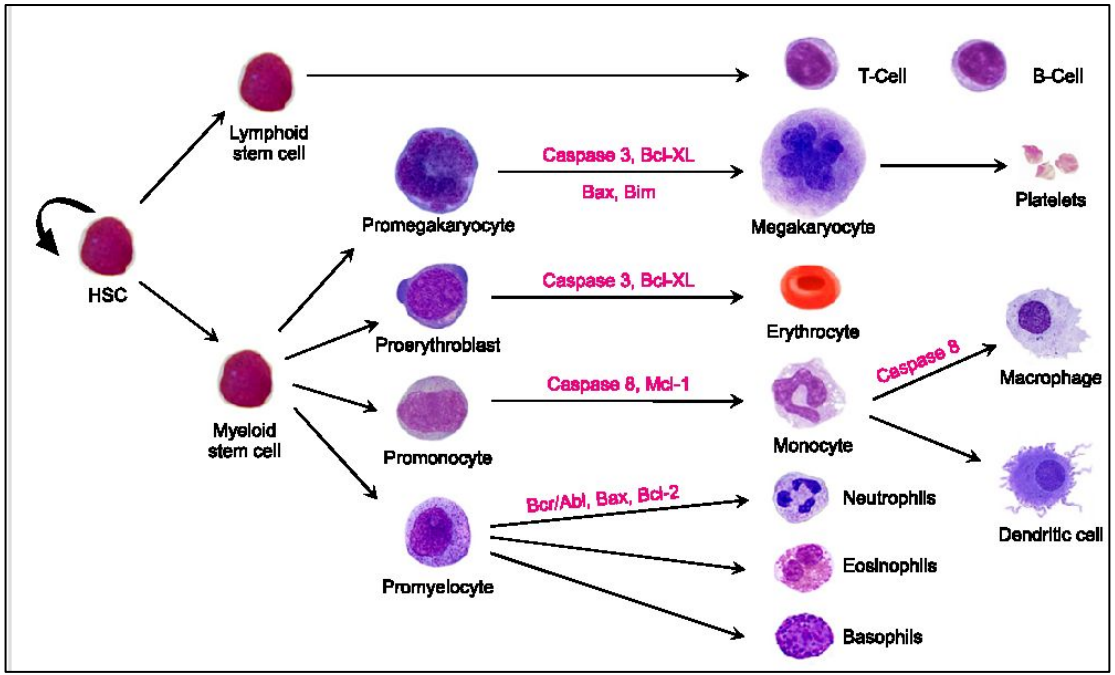
- Malignant multiple sclerosis (MS) is a rare but clinically important subtype of MS- patients reach significant level of disability in a short period of time
- Multiple sclerosis (MS) is a potentially disabling disease of the brain and spinal cord (central nervous system).
- In MS, the immune system attacks the protective sheath (myelin) that covers nerve fibers and causes communication problems between your brain and the rest of your body. Eventually, the disease can cause permanent damage or deterioration of the nerves.
- the mainstay of their treatment is plasmapheresis or immunosuppression with mitoxantrone, cyclophosphamide, cladribine or, lately, bone marrow transplantation.
- A report on the case of a 17-year old patient with malignant MS who was treated with high-dose chemotherapy plus anti-thymocyte globulin followed by autologous stem cell transplantation.- resulted in an impressive and long-lasting clinical and radiological response

4. **Parkinson’s Disease**

- **Parkinson's disease** (**PD**), or simply **Parkinson's**,is a long-term degenerative disorder of the central nervous system that mainly affects the motor system
- The most obvious early symptoms are tremor, rigidity, slowness of movement, and difficulty with walking. Cognitive and behavioral problems may also occur with depression, anxiety, and apathy
- The motor symptoms of the disease result from the death of cells in the substantia nigra, a region of the midbrain, leading to a dopamine deficit.
- Doctors firstly isolated adult stem cells from the patient’s brain, they were then cultured in vitro and encouraged to turn into dopamine-producing neurons. As soon as tests showed that the cells were producing dopamine they were then re-injected into the man’s brain. After the transplant, the man’s condition was seen to improve and he experienced a reduction in the trembling and muscle rigidity associated with the disease. Brain scans taken 3-months after the transplant revealed that dopamine production had increased by 58%, however it later dropped but the Parkinson’s symptoms did not return. The study is the first human study to show that stem cell transplants can help to treat Parkinson’s.

2. Hematopoietic disorder

- **Hematologic diseases** are disorders of the blood and blood-forming organs. It include problems with red blood cells, white blood cells, platelets, bone marrow, lymph nodes, and spleen
- In addition to blood cell cancers, **hematologic diseases** include rare genetic **disorders**, anemia, conditions related to HIV, sickle cell **disease**, and complications from chemotherapy or transfusions.



List of Hematopoietic disorders

Myeloid

- **Hemoglobinopathies** (congenital abnormality of the hemoglobin molecule or of the rate of hemoglobin synthesis)
  - Sickle cell disease
  - Thalassemia
  - Methemoglobinemia
- **Anemias** (lack of red blood cells or hemoglobin)
  - Iron-deficiency anemia
  - Megaloblastic anemia
    - Vitamin B_{12} deficiency
      - Pernicious anemia
    - Folate deficiency
  - Hemolytic anemias (destruction of red blood cells)
  - Hemoglobinopathies (where these is an unstable or crystalline hemoglobin)
- **Decreased numbers of cells**
  - Myelodysplastic syndrome
  - Myelofibrosis
  - Neutropenia (decrease in the number of neutrophils)
  - Agranulocytosis
  - Glanzmann's thrombasthenia
  - Thrombocytopenia (decrease in the number of platelets)
  -