
# Training Manual

# Index

# I Basic Anatomy

## I A Cells, Tissues & Organs

### I AA Cells

**Introduction**

Cells are the structural and functional units of all living organisms. It can take in nutrients, convert these nutrients into energy, carry out specialized functions, and reproduce as necessary. Even more amazing is that each cell stores its own set of instructions for carrying out each of these activities. Humans have an estimated 100,000,000,000,000 cells!

**Cell structures**

The cell is made up of a number of organelles (small organs) or structures. We shall focus our attention on a few organells and understand their functions

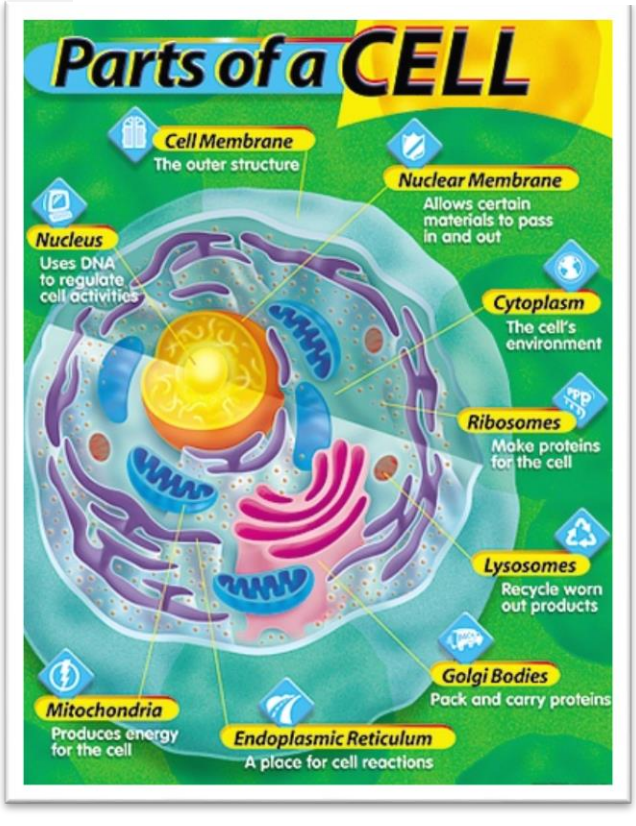



Fig 1 : Components of a typical human cell

*Plasma Membrane—the cell's protective coat*

The outer lining of the cell is called the plasma membrane. This membrane serves to separate and protect a cell from its surrounding environment and is made mostly from a double layer of proteins and lipids, fat-like molecules.

*Cytoplasm—the cell's inner space*

Inside the cell there is a large fluid-filled space called the cytoplasm (cytosol). The cytosol contains dissolved nutrients, helps break down waste products, and moves material around the cell. The cytoplasm also contains many salts and is an excellent conductor of electricity, creating the perfect environment for the mechanics of the cell.

*Nucleus—the cell's center*

The nucleus is the most conspicuous organelle and it houses the cell's chromosomes and is the place where almost all DNA replication and RNA synthesis occur.

*Genetic Material*

Two different kinds of genetic material exist:

- deoxyribonucleic acid (DNA) and
- ribonucleic acid (RNA).

Most organisms are made of DNA, but a few viruses have RNA as their genetic material. The biological information contained in an organism is encoded in its DNA or RNA sequence.

*Ribosome—the protein production machine*

The ribosome is a large complex composed of many molecules, including RNAs and proteins, and is responsible for processing the genetic instructions.

*Mitochondria and Chloroplasts—the power generators*

Mitochondria are the cell's power producers. They convert energy into forms that are usable by the cell.

*Lysosomes and Peroxisomes—the cellular digestive system*

Lysosomes and peroxisomes are often referred to as the garbage disposal system of a cell. One function of a lysosome is to digest foreign bacteria that invade a cell. Other functions include helping to recycle receptor proteins and other membrane components and degrading worn out organelles such as mitochondria. Lysosomes can even help repair damage to the plasma membrane by serving as a membrane patch, sealing the wound. Peroxisomes function to rid the body of toxic substances, such as hydrogen peroxide, or other metabolites and contain enzymes concerned with oxygen utilization. High numbers of peroxisomes can be found in the liver, where toxic byproducts are known to accumulate.

*Endoplasmic Reticulum-macromolecule manager*

The endoplasmic reticulum (ER) is the transport network for molecules targeted for certain modifications and specific destinations, as compared to molecules that will float freely in the cytoplasm.

## I AB Tissues

Cells group together in the body to form tissues - a collection of similar cells that group together to perform a specialized function. There are four primary tissue types in the human body: epithelial tissue, connective tissue, muscle tissue and nerve tissue.

- *Epithelial tissue* - The cells of epithelial tissue pack tightly together and form continuous sheets that serve as linings in different parts of the body. Epithelial tissue serves as membranes lining organs and helping to keep the body's organs separate, in place and protected. Some examples of epithelial tissue are the outer layer of the skin, the inside of the mouth and stomach, and the tissue surrounding the body's organs.
- *Connective tissue* - There are many types of connective tissue in the body. Generally speaking, connective tissue adds support and structure to the body. Most types of connective tissue contain fibrous strands of the protein collagen that add strength to connective tissue. Some examples of connective tissue include the inner layers of skin, tendons, ligaments, cartilage, bone and fat tissue. In addition to these more recognizable forms of connective tissue, blood is also considered a form of connective tissue.
- *Muscle tissue* - Muscle tissue is a specialized tissue that can contract. Muscle tissue contains the specialized proteins actin and myosin that slide past one another and allow movement. Examples of muscle tissue are contained in the muscles throughout your body.
- *Nerve tissue* - Nerve tissue contains two types of cells: neurons and glial cells. Nerve tissue has the ability to generate and conduct electrical signals in the body. These electrical messages are managed by nerve tissue in the brain and transmitted down the spinal cord to the body.

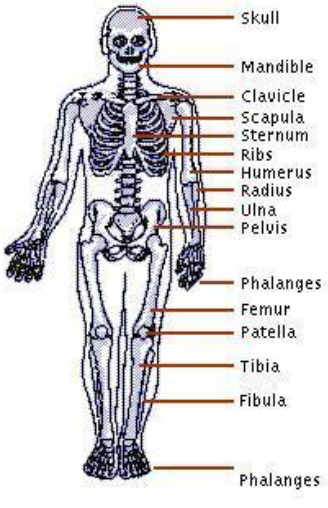
## I AC Organs

Organs are the next level of organization in the body. An organ is a structure that contains at least two different types of tissue functioning together for a common purpose. There are many different organs in the body: the liver, kidneys, heart, even your skin is an organ.

**Organ systems**

Organ systems are composed of two or more different organs that work together to provide a common function. There are ten major organ systems in the human body. They have been described below:

***Skeletal System***



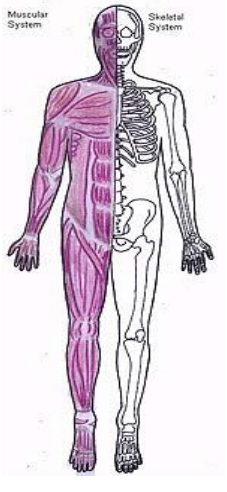

Major role:

The main role of the skeletal system is to provide support for the body, to protect delicate internal organs and to provide attachment sites for the organs.

Major organs:

Bones, cartilage, tendons and ligaments.

***Muscular System***



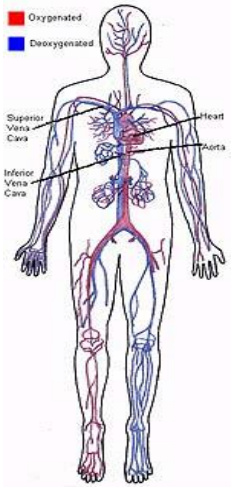

Major role:

The main role of the muscular system is to provide movement. Muscles work in pairs to move limbs and provide the organism with mobility. Muscles also control the movement of materials through some organs, such as the stomach and intestine, and the heart and circulatory system.

Major organs:

Skeletal muscles and smooth muscles throughout the body.

***Circulatory System***



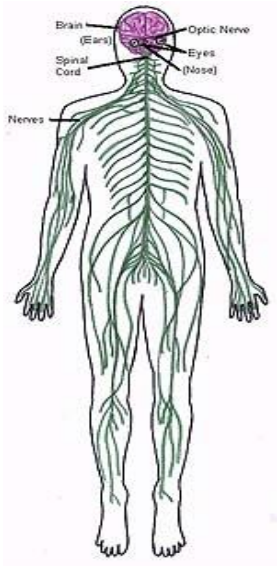

Major role:

The main role of the circulatory system is to transport nutrients, gases (such as oxygen and $CO_2$), hormones and wastes through the body.

Major organs:

Heart, blood vessels and blood.

***Nervous System***



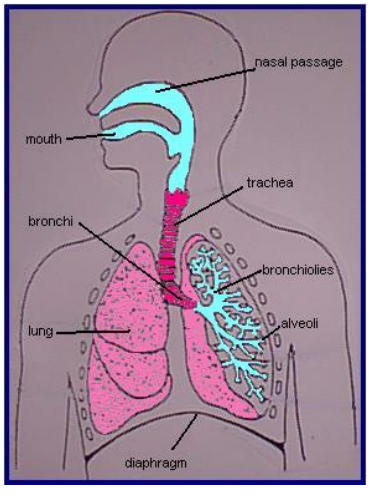

Major role:

The main role of the nervous system is to relay electrical signals through the body. The nervous system directs behaviour and movement and, along with the endocrine system, controls physiological processes such as digestion, circulation, etc.

Major organs:

Brain, spinal cord and peripheral nerves.

***Respiratory System***



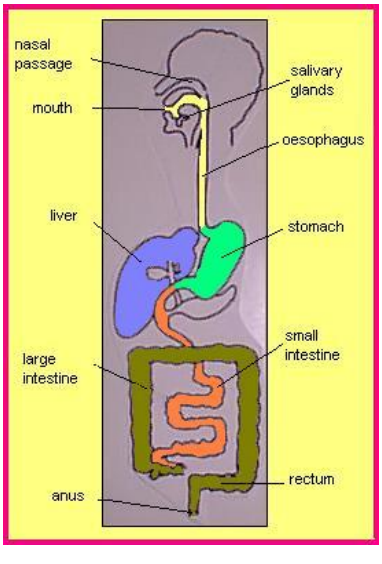

Major role:

The main role of the respiratory system is to provide gas exchange between the blood and the environment. Primarily, oxygen is absorbed from the atmosphere into the body and carbon dioxide is expelled from the body.

Major organs:

Nose, trachea and lungs.

***Digestive System***



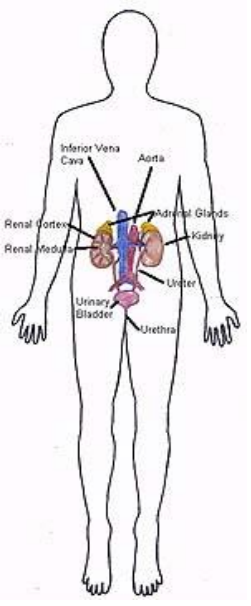

Major role:

The main role of the digestive system is to breakdown and absorbs nutrients that are necessary for growth and maintenance.

Major organs:

Mouth, esophagus, stomach, small and large intestines.

***Excretory System***



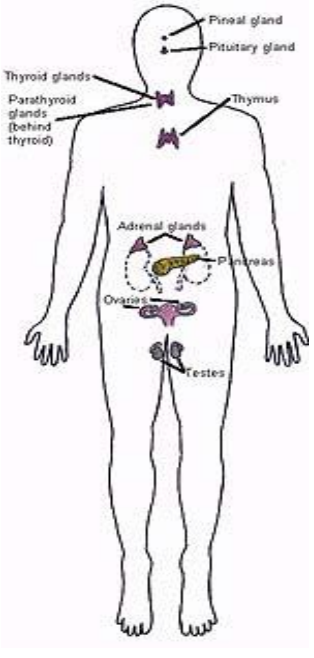

Major role:

The main role of the excretory system is to filter out cellular wastes, toxins and excess water or nutrients from the circulatory system.

Major organs:

Kidneys, ureters, bladder and urethra.

***Endocrine System***



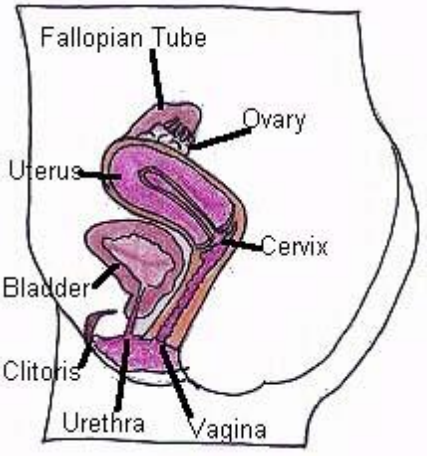

Major role:

The main role of the endocrine system is to relay chemical messages through the body. In conjunction with the nervous system, these chemical messages help control physiological processes such as nutrient absorption, growth, etc.

Major organs:

Many glands exist in the body that secretes endocrine hormones. Among these are the hypothalamus, pituitary, thyroid, pancreas and adrenal glands.

***Reproductive System***



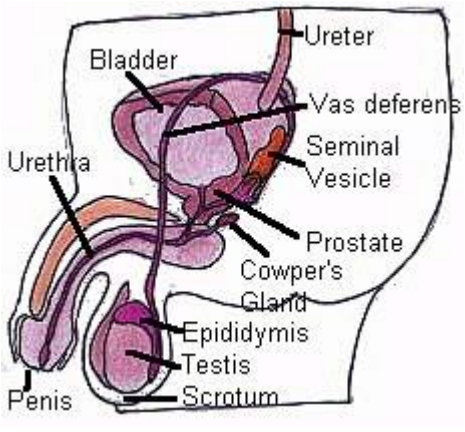

Major role:

The main role of the reproductive system is to manufacture cells that allow reproduction. In the male, sperm are created to inseminate egg cells produced in the female.

Major organs:

Female (top right): ovaries, oviducts, uterus, vagina and mammary glands.

Male (bottom right): testes, seminal vesicles and penis.




***Lymphatic/ Immune System***

Major role:

The main role of the immune system is to destroy and remove invading microbes and viruses from the body. The lymphatic system also removes fat and excess fluids from the blood.

Major organs:

Lymph, lymph nodes and vessels, white blood cells, T- and B- cells.

# I Basic Anatomy

## I B Anatomy of the tooth

**Introduction**

Teeth (singular tooth) are small, calcified, whitish structures found in the jaws (or mouths) that are used to break down food. Some animals, particularly carnivores, also use teeth for hunting or for defensive purposes. The roots of teeth are covered by gums. Teeth are not made of bone, but rather of multiple tissues of varying density and hardness.

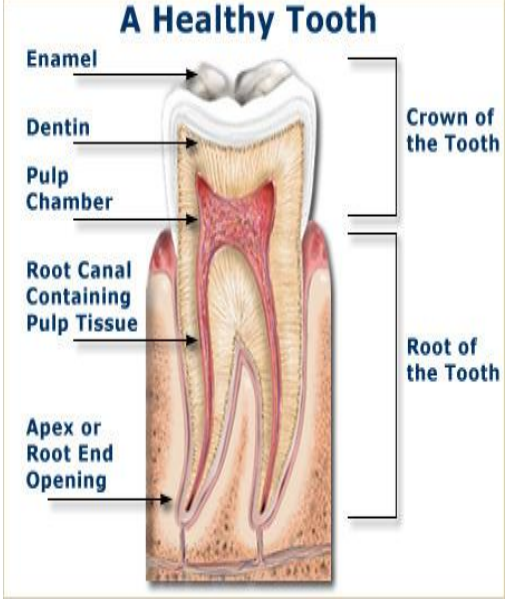
Mammals are diphyodont, meaning that they develop two sets of teeth. In humans, the first set (the "baby," "milk," "primary" or "deciduous" set) normally starts to appear at about six months of age, although some babies are born with one or more visible teeth, known as neonatal teeth. Normal tooth eruption at about six months is known as teething and can be painful.

**Parts of a tooth**

A tooth can be divided into the following parts:

*Enamel*

Enamel is the hardest and most highly mineralized substance of the body. It is one of the four major tissues which make up the tooth, along with dentin, cementum, and dental pulp. It is normally visible and must be supported by underlying dentin. Ninety-six percent of enamel consists of mineral, with water and organic material comprising the rest. The normal color of enamel varies from light yellow to grayish white. At the edges of teeth where there is no dentin underlying the enamel, the color sometimes has a slightly blue tone.




Enamel's primary mineral is hydroxylapatite, which is a crystalline calcium phosphate. The large amount of minerals in enamel accounts not only for its strength but also for its brittleness.

*Dentine*

Dentin is the substance between enamel or cementum and the pulp chamber. It is secreted by the odontoblasts of the dental pulp. The porous, yellow-hued material is made up of 70% inorganic materials, 20% organic materials, and 10% water by weight. Dentin is a mineralized connective tissue with an organic matrix of collagenous proteins. Because it is softer than enamel, it decays more rapidly and is subject to severe cavities if not properly treated, but dentin still acts as a protective layer and supports the crown of the tooth.

*Cementum*

Cementum is a specialized bone like substance covering the root of a tooth. It is approximately 45% inorganic material (mainly hydroxyapatite), 33% organic material (mainly collagen) and 22% water. Cementum is excreted by cementoblasts within the root of the tooth and is thickest at the root apex. Its coloration is yellowish and it is softer than either dentin or enamel. The principal role of cementum is to serve as a medium by which the periodontal ligaments can attach to the tooth for stability.

*Pulp*

The dental pulp is the central part of the tooth filled with soft connective tissue. This tissue contains blood vessels and nerves that enter the tooth from a hole at the apex of the root. Along the border between the dentin and the pulp are odontoblasts, which initiate the formation of dentin. Other cells in the pulp include fibroblasts, preodontoblasts, macrophages and T lymphocytes. The pulp is commonly called "the nerve" of the tooth.

*Periodontal ligaments*

The periodontal ligament is a specialized connective tissue that attaches the cementum of a tooth to the alveolar bone. This tissue covers the root of the tooth within the bone. The functions of the periodontal ligaments include attachment of the tooth to the bone, support for the tooth, formation and resorption of bone during tooth movement, sensation, and eruption.

*Alveolar bone*

The alveolar bone is the bone of the jaw which forms the alveolus around teeth. Like any other bone in the human body, alveolar bone is modified throughout life. Osteoblasts create bone and osteoclasts destroy it, especially if force is placed on a tooth. As is the case when movement of teeth is attempted through orthodontics, an area of bone under compressive force from a tooth moving toward it has a high osteoclast level, resulting in bone resorption. An area of bone receiving tension from periodontal ligaments attached to a tooth moving away from it has a high number of osteoblasts, resulting in bone formation.

*Gingiva*

The gingiva ("gums") is the mucosal tissue that overlays the jaws. There are three different types of epithelium associated with the gingiva: gingival, junctional, and sulcular epithelium. These three types form from a mass of epithelial cells known as the epithelial cuff between the tooth and the mouth.

**Types of teeth**

As discussed above, humans have two set of dentition. They are the primary dentition (teeth) and the permanent dentition (teeth).

*Primary teeth*

Among deciduous (primary) teeth, ten are found in the maxilla (upper jaw) and ten in the mandible (lower jaw), for a total of 20. In the primary set of teeth, there are two types of incisors - centrals and laterals, a canine and two types of molars - first and second. All primary teeth are normally later replaced with their permanent counterparts.

*Permanent teeth*

Among permanent teeth, 16 are found in the maxilla and 16 in the mandible, for a total of 32. The permanent tooth in each jaw consists of 2 incisors, 1 canine, 2 premolars and 3 molars. Thus, the maxillary teeth are the maxillary central incisor, maxillary lateral incisor, maxillary canine, maxillary first premolar, maxillary second premolar, maxillary first molar, maxillary second molar, and maxillary third molar. Likewise, the mandibular teeth are the mandibular central incisor, mandibular lateral incisor, mandibular canine, mandibular first premolar, mandibular second premolar, mandibular first molar, mandibular second molar, and mandibular third molar. Third molars are commonly called "wisdom teeth" and may never erupt into the mouth or form at all. If any additional teeth form, for example, fourth and fifth molars, which are rare, they are referred to as supernumerary teeth (hyperdontia). Development of fewer than the usual number of teeth is called hypodontia.

# II Stem Cells

## II A Basics of Stem Cells

**Introduction**

Stem cells are the "master" cells of the body. Stem cells have two important characteristics that distinguish them from other types of cells. First, they are unspecialized cells that renew themselves for long periods through cell division. The second is that under certain physiologic or experimental conditions, they can be induced to become cells with special functions such as the beating cells of the heart muscle or the insulin producing cells of the pancreas. It has been hypothesized by scientists that stem cells may become the basis for treating diseases such as Parkinson's disease, diabetes, and heart disease.

**Properties of stem cells**

Stem cells differ from other kinds of cells in the body. All stem cells—regardless of their source—have three general properties:

- they are unspecialized;
- they are capable of dividing and renewing themselves for long periods; and
- they can give rise to specialized cell types.

*Stem cells are unspecialized*

One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures that allow it to perform specialized functions. A stem cell cannot work with its neighbors to pump blood through the body (like a heart muscle cell); it cannot carry molecules of oxygen through the bloodstream (like a red blood cell); and it cannot fire electrochemical signals to other cells that allow the body to move or speak (like a nerve cell). However, unspecialized stem cells can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells.

*Stem cells are capable of dividing and renewing themselves for long periods*

Unlike muscle cells, blood cells, or nerve cells—which do not normally replicate themselves—stem cells may replicate many times. When cells replicate themselves many times over it is called proliferation. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells. If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are said to be capable of long-term self-renewal.

*Stem cells can give rise to specialized cells*

When unspecialized stem cells give rise to specialized cells, the process is called differentiation.

**Types of stem cells**

Stem cells can be broadly divided into three types depending upon their origin:

- *embryonic stem cells* – these cells are derived from the embryo during the early developmental stages
- *adult stem cells* – these stem cells are present in children and adults throughout the body after embryonic development is complete
- *induced pluripotent stem cells* – these cells are artificially derived from a non-pluripotent cell by inducing a forced expression of specific genes

Further depending upon the kind of cells they would differentiate into, stem cells can be further classified as:

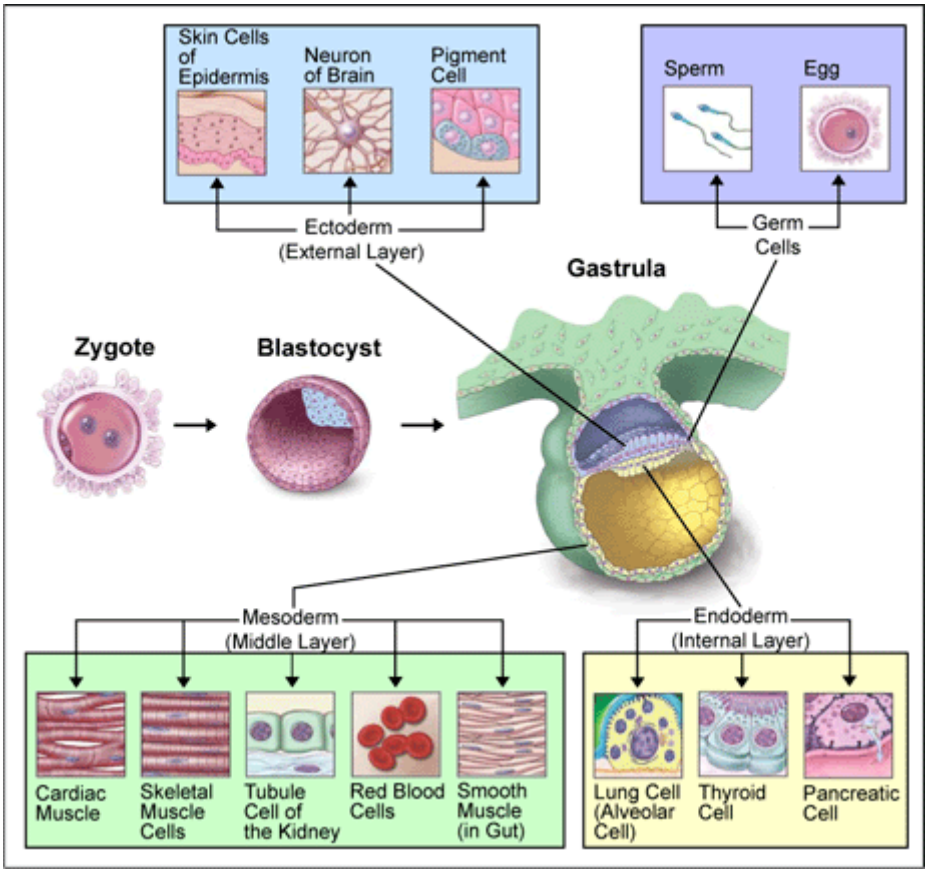
- *hematopoietic stem cells* – these are blood related stem cells and give rise to all the blood cells of our body such as platelets, RBCs, WBCs etc.
- *mesenchymal stem cells* – these are tissue related cells and give rise to all the other tissues and organs of our body such as hair, skin, eyes, teeth, bone, heart, lungs, kidneys etc.

**Potency**

Stem cells are categorized by their potential to differentiate into other types of cells. Embryonic stem cells are the most potent since they must become every type of cell in the body. The full classification includes:

- *totipotent* - the ability to differentiate into all possible cell types. Examples are the zygote formed at egg fertilization and the first few cells that result from the division of the zygote.
- *pluripotent* - the ability to differentiate into almost all cell types. Examples include embryonic stem cells and cells that are derived from the mesoderm, endoderm, and ectoderm germ layers that are formed in the beginning stages of embryonic stem cell differentiation.
- *multipotent* - the ability to differentiate into a closely related family of cells. Examples include hematopoietic (adult) stem cells that can become red and white blood cells or platelets.
- *oligopotent* - the ability to differentiate into a few cells. Examples include (adult) lymphoid or myeloid stem cells.
- *unipotent* - the ability to only produce cells of their own type, but have the property of self-renewal required to be labeled a stem cell. Examples include adult muscle stem cells.

Embryonic stem cells are considered pluripotent instead of totipotent because they do not have the ability to become part of the extra-embryonic membranes or the placenta.




**Induced pluripotent stem cells** (iPSCs) are adult cells that have been genetically reprogrammed to an embryonic stem cell-like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells.

Although additional research is needed, iPSCs are already useful tools for drug development and modeling of diseases, and scientists hope to use them in transplantation medicine. Viruses are currently used to introduce the reprogramming factors into adult cells, and this process must be carefully controlled and tested before the technique can lead to useful treatments for humans. In animal studies, the virus used to introduce the stem cell factors sometimes causes cancers. Researchers are currently investigating non- viral delivery strategies. In any case, this breakthrough discovery has created a powerful new way to “de-differentiate” cells whose developmental fates had been previously assumed to be determined. In addition, tissues derived from iPSCs will be a nearly identical match to the cell donor and thus probably avoid rejection by the immune system. The iPSC strategy creates pluripotent stem cells that, together with studies of other types of pluripotent stem cells, will help researchers learn how to reprogram cells to repair damaged tissues in the human body.

# II Stem Cells

## II B Adult Stem Cells

**Introduction**

An adult stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ, can renew itself, and can differentiate to yield the major specialized cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. Some scientists now use the term somatic stem cell instead of adult stem cell. Unlike embryonic stem cells, which are defined by their origin (the inner cell mass of the blastocyst), the origin of adult stem cells in mature tissues is unknown.

The history of research on adult stem cells began about 40 years ago. In the 1960s, researchers discovered that the bone marrow contains at least two kinds of stem cells. One population, called hematopoietic stem cells, forms all the types of blood cells in the body. A second population, called bone marrow stromal cells, was discovered a few years later. Stromal cells are a mixed cell population that generates bone, cartilage, fat, and fibrous connective tissue.
Also in the 1960s, scientists who were studying rats discovered two regions of the brain that contained dividing cells, which become nerve cells. Despite these reports, most scientists believed that new nerve cells could not be generated in the adult brain. It was not until the 1990s that scientists agreed that the adult brain does contain stem cells that are able to generate the brain's three major cell types — astrocytes and oligodendrocytes, which are non-neuronal cells, and neurons, or nerve cells.

**Source of adult stem cells**

Over the years, scientists have identified adult stem cells from a number of different tissues and parts of the body. Some of the important sources include:

- bone marrow,
- liver,
- brain,
- pancreas,
- umbilical cord blood and tissue,
- teeth

## Comparison between the sources of Stem cells

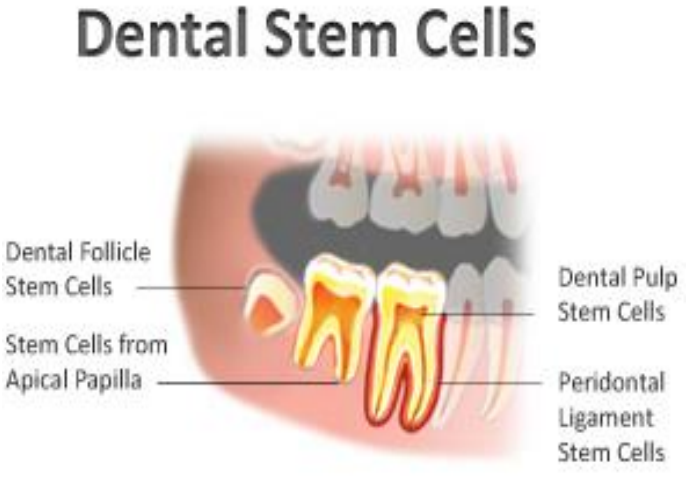
| | CORD BLOOD | BONE MARROW | DENTAL PULP STEM CELL | MENSTRUAL STEM CELLS |
|---|---|---|---|---|
| **STEM CELL RELATED** | | | | |
| **STEM CELL TYPE** | Adult stem cell : primarily Haematopoetic, with small amounts of early mesenchymal and other cells | Adult Stem cell : Haematopoetic & Mesenchymal | Adult Stem cell : Mesenchymal stem cells | Adult Stem cell: Mesenchymal Stem cells |
| **STEM CELL POTENCY** | Multipotent | Multipotent | Multipotent | Multipotent |
| **STEM CELL QUALITY : POPULATION DOUBLING** | Cannot be cultured / expanded | 30-50 | SHED > 140<br>DPSC: 60-120 | 25-68 |
| **THERAPY APPLICATIONS** | Primarily blood related disorders | Both blood & connective tissue related disorders | Connective tissue related disorders | Connective tissue related diorders |
| **STAGE OF RESEARCH** | Approved for blood related disorders | Approved for blood related disorders | Clinical trial stage for bone regeneration | Animal studies going on |
| **BANKING RELATED** | | | | |
| **COLLECTION FREQUENCY OPTIONS** | Only women, once in a lifetime, restrictive only during birth, limited chances | Multiple | Everyone - Multiple<br>Baby tooth : 12 teeth, wisdom tooth (2 molars - minimum) | Restrictive to women, but multiple options 13 - 47 yrs. Preferable before the age of 30 yrs |
| **COLLECTION PROCEDURE** | Painless, less invasive | Invasive and can be quite painful - Collection performed with large needle into hip | Painless, less invasive | Painless, but uncomfortable, time consuming |
| **RISK TO DONOR** | None | Uncommon (anesthesia related, surgical complications, emergency blood transfusion, etc.) | None | Uncomfortable, time consuming |
| **RISK TO INFECTION (SAFETY)** | None | None | None | Very high |
| **TIME TO ACQUIRE** | Days - Units are pre-harvested, and can be issued at a short notice | Difficulty at times tracking down donors who registered, median search time is greater than four months | Days - Units are preharvested | Days - Units are preharvested |
| **ACCESS FOR TRANSPLANT** | Easy - Can be pulled from inventory and shipped within 24 hours | Difficult - Donor has to coordinate time off work, transplant team and hospital scheduling must align | Easy - Can be pulled from inventory and shipped within 24 hours | Easy - Can be pulled from inventory and shipped within 24 hours |

## II Stem Cells

## II C Dental Stem Cells

Dental stem cells are stem cells that are harvested/ obtained from teeth and its supporting structures. Thus the various parts of a tooth from which stem cells can be obtained are:

- Dental-pulp complex (DPSC)
- Stem cells from Human Exfoliated Deciduous teeth (SHED)
- Periodontal Ligament Stem Cell (PDLSC)
- Dental follicle stem cell (DFSC)
- Stem cells of Apical Papilla (SCAP)




**Benefits of banking dental stem cells**

- Dental pulp is a remarkable site of stem cells
- Collecting stem cells from dental pulp is a non-invasive practice that can be performed in children, adolescents as well as adults
- Tissue sacrifice is very low when collecting dental pulp stem cells
- Several different cell types including heart cells, bone cells, cartilage cells, nerve cells amongst others can be obtained from dental pulp stem cells owing to their multi-potency nature
- These dental stem cells can be multiplied more than 100 times and this is an enormous advantage when compared to other sources of stem cells
- Dental pulp is an ideal source for tissue engineering and for clinical use in several pathologies requiring bone tissue growth and repair
- Dental pulp stem cells can be cryo-preserved (banked) and stored for long periods
- If bone marrow is the site of first choice for blood related (hematopoietic) stem cell collection, dental pulp must be considered one of the major sites for tissue related (mesenchymal) cell collection.
- Dental pulp presents an opportunity to safely, inexpensively and painlessly acquire stem cells and save these valuable stem cells in case they are ever needed

**Banking of stem cells at an early age**

Like it or not, aging is a part of all living beings. There is more to aging than having gray hair and wrinkles on the skin. Scientifically,it is not we as individuals who age instead it is the cells in our body that age. Thus, as we age, the heart muscle pumps less efficiently, bones shrink in size and density, muscles and joints lose their strength and flexibility, skin becomes thin and less elastic, kidney and liver functions begin to decrease. This happens because the cells in these tissues and organs undergo wear and tear with age. Cells are the basic building blocks of tissues. All cells experience changes with aging and that includes your stem cells. They become larger and are less able to divide and reproduce. Many cells lose their ability to function or they begin to function abnormally. Some of these cells may also undergo cancerous changes with age. Hence, if one has to utilize the full potential of the function of stem cell then these cells have to be banked early.

# III Stem Cell Applications

**Applications of stem cells**

Stem cells have potential uses in many different areas of research and medicine, as described below.

**Replacing damaged tissue**

Human stem cells could be used in the generation of cells and tissues for cell-based therapies. This involves treating patients by transplanting specialised cells that have been grown from stem cells in the laboratory.

Due to their ability to replace damaged cells in the body, stem cells could be used to treat a range of conditions including heart failure, spinal injuries, diabetes and Parkinson disease. Scientists hope that transplantation and growth of appropriate stem cells in damaged tissue will regenerate the various cell types of that tissue. This forms the basis for the new era in medicine called Regenerative Medicine

**Studying human development**

Stem cells could be used to study early events in human development and find out more about how cells differentiate and function. This may help researchers find out why some cells become cancerous and how some genetic diseases develop. This knowledge may lead to clues about how these diseases may be prevented.

**Testing new drugs**

Stem cells grown in the laboratory may be useful for testing drugs and chemicals before they are used in humans. The cells could be directed to differentiate into the cell types that are important for screening that drug. These cells may be more likely to mimic the response of human tissue to the drug being tested than animal models do. This may make drug testing safer, cheaper and more ethically acceptable to those who oppose the use of animals in pharmaceutical testing.

**Screening toxins**

Stem cells may be useful for screening potential toxins in substances such as pesticides before they are used in the environment.

**Testing gene therapy methods**

Stem cells may prove useful during the development of new methods for gene therapy that may help people suffering from genetic illnesses.

However, the most significant use of stem cells would be its application in various disease conditions to regenerate cells and replace damaged cells. A few important applications of stem cells with a brief introduction to the disease condition are described below.

**Regenerative Medicine**

Regenerative medicine is the "process of replacing or regenerating human cells, tissues or organs to restore or establish normal function". This field holds the promise of regenerating damaged tissues and organs in the body by replacing damaged tissue and/or by stimulating the body's own repair mechanisms to heal previously irreparable tissues or organs. Regenerative medicine also empowers scientists to grow tissues and organs in the laboratory and safely implant them when the body cannot heal itself. Importantly, regenerative medicine has the potential to solve the problem of the shortage of organs available for donation compared to the number of patients that require life-saving organ transplantation, as well as solve the problem of organ transplant rejection, since the organ's cells will match that of the patient. This field of medicine holds promise for degenerative disease, a disease in which the function or structure of the affected tissues or organs will progressively deteriorate over time, whether due to normal bodily wear or lifestyle choices such as exercise or eating habits or due to autoimmune responses..

The goal of stem cell therapy is to repair a damaged tissue that can't heal itself. This might be accomplished by transplanting stem cells into the damaged area and directing them to grow to a new, healthy tissue. It may also be possible to 'coax' stem cells already in the body to work overtime and produce new tissue. To date, researchers have found more success with stem cell transplants. Considering the immense interest worldwide, it comes as no surprise that the global market for stem cell therapy is expected to be $20 billion by 2010, as per a Frost & Sullivan study. There are almost 180 prominent companies working on stem cell research in the world, majority of which is based in the US, followed by the EU, Israel, Thailand, Canada, and Australia.

# Detailed list of stem cell uses

Reference BRITISH MEDICAL JOURNAL 2000; 321:433 - 437 & OTHER SOURCES

| **Allogenic uses: (matching donor cells)** | **Autologous uses: (own cells)** |
|---|---|
| **ESTABLISHED USES** | |
| • Severe aplastic anaemia | • Acute lymphoblastic leukaemia (certain subtypes) |
| • Acute myeloid leukaemia in first complete remission (patient < 50 years old) | • Non-Hodgkin's lymphoma in second complete remission |
| • Myelodysplasia (patient < 50 years old) | • Multiple myeloma |
| • Acute lymphoblastic leulaemia in first complete remission (certain subtypes) | • Solid tumours such as neuroblastoma and testicular cancer |
| • Severe congenital immunodeficiency | • Aplastic anaemia (banked cells only) |
| • Acute myeloid leukaemia and acute lymphoblastic leukaemia in second complete remission | • Hodgkin's disease in second complete remission |
| • Thalassaemia | |
| • Chronic myeloid leukaemia | |
| **EMERGING USES** | |
| • Multiple myeloma | • Chronic lymphoblastic leukaemia |
| • Hodgkin's disease | • Acute myeloid leukaemia |
| • Non-Hodgkin's disease | • Solid tumours, such as breast, ovarian |
| • Haemoglobinopathies including: | • Chronic myeloid leukaemia |
| • Sickle cell anaemia | • Hodgkin's disease in first complete |

- Thalassemia
- Multiple others
- Inborn errors of metabolism including:
- Osteopetrosis
- ALL amyloidsis
- Multiple others

remission

- Non-Hodgkin's lymphoma in first complete remission
- Systemic sclerosis
- Autoimmune pulmonary hypertension
- Necrotizing vasculititis
- Rheumatoid arthritis: resistant or complicated
- Systematic lupus erthematosus
- Antiphospholipid syndrome
- Cryoglobulinaemia
- Systemic sclerosis variants with pulmonary fibrosis
- Dermatomyositis
- Necrotizing vasculititis
- Multiple sclerosis
- Myasthenia gravis
- Autoimmune thrombocytopenia
- Autoimmune haemolytic anaemia
- Autoimmune neutropenia
- Inflammatory bowel disease
- Autoimmune diabetes mellitus

**EXPERIMENTAL USES**

- Chronic lymphocytic leukaemia
- Renal cell carcinoma
- Breast cancer
- Amyloidosis
- Other solid tumours
- Juvenile chronic arthritis

## III Stem Cell Applications

## III B Key Applications – Diabetes

Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar levels (fasting glucose levels more than120).

**Major types of diabetes:**

• Type 1 diabetes: results from the body's failure to produce insulin, and presently requires the person to inject insulin. (Also referred to as insulin-dependent diabetes mellitus, IDDM for short, and juvenile diabetes.)

• Type 2 diabetes: results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. (Formerly referred to as non-insulin-dependent diabetes mellitus, NIDDM for short, and adult-onset diabetes.)

**Causes:**

• Loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency. The majority of type 1 diabetes is of the immune-mediated nature, where beta cell loss is a T-cell mediated autoimmune attack

• Insulin resistance - The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor

• Increase in the hepatic glucose output by the liver

**Current Treatment:**

• Type I diabetes:

Pancreas transplants – tried with limited success

**Clinical Situation: No cure only management**

**Current Management:**

• Type I diabetes: along with OHAs

Insulin given subcutaneously, either by injections or by insulin pump

• Type II diabetes: OHAs: Single or combined based on the problem; mostly combination

Biguanides (Metformin): reduce hepatic glucose output & increase uptake of glucose by peripheral tissues

Glitazones (Pioglitazone): influence insulin sensitive genes, leading to better use of glucose by the cells

Sulphonylureas(Glimepride): stimulate endogenous release of insulin

**Stem cell therapy: Proposed cure and success**

Current successes: Human studies India:

**Study 1**

Organization: IKRDC

Year of Study: 2009

Doctors: Dr. H.L. Trivedi; Dr. Aruna Vaniker

No of patients: 5 diabetes mellitus patients

Study: Human Adipose tissue derived mesenchymal stem cells combined with hematopoietic stem cell transplantation synthesize insulin

Conclusion: All patients were successfully infused CBM plus h-AD-MSC without any untoward effects and showed 30% to 50% decreased insulin requirements with 4- to 26- fold increased serum c-peptide levels, with a mean follow-up of 2.9 months.

**Study 2**

Organization: Stempeutics

Year of Study: 2009

Details: Stempeutics received DCGI clearance for the company's Investigational

Medicinal Product - "Stempeucel" for Phase II clinical trials for four debilitating diseases- It is a multicentric, placebo controlled, double blind, allogeneic clinical trials addressing Osteo Arthritis, Liver Cirrhosis, Diabetes Mellitus type 2 and Chronic Obstructive Pulmonary Disease- Stempeutics established large scale production of Mesenchymal Stem Cells for Clinical Trials using a patented upscaling process- Based on the successful outcome of clinical studies plans to introduce the FIRST stem cell based drug – available off the shelf in India by end of 2013.

**Study 3**

Organization: Global hospitals

Year of Study: 2009

Details: Global Hospitals to use stem cells to cure diabetes By Sangeetha G. Dec 13 2009 , Chennai

Global Hospitals is set to become the first hospital in India to develop and commercially use stem cells for treating type-I diabetes patients by June next year. Global Hospitals has been working for the past two years on using mesenchymal cells isolated from cord blood to create pancreatic cells capable of producing insulin inside the human body. These stem cells can be transplanted in patients with type-I diabetes, who either do not produce or secrete insufficient amount of insulin. “The study will be completed in March 2010 and in another three months we will seek necessary approval from Indian Council for Medical Research and Drug Controller General of India and conduct transplants in patients, though in limited numbers initially,” said C Emmanuel, director, academics and research, Global Hospitals.

**Global:**

**Study 1**

Organization: University of Sao Paulo & Northwestern University of Chicago

Doctors: Dr. Julio Voltarelli

No of patients: 15 diabetes mellitus patients

Study: Bone Marrow derived stem cells

Conclusion: The group first reported its initial achievement in 2007, with 15 type 1 diabetes patients who received their own stem cells and no longer needed insulin to control their blood sugar levels. In the new study, a follow-up of their previous work, Voltarelli and his colleagues detailed the same success with an additional eight patients, and also confirmed that in the majority of them, the stem cell transplant led to an appreciable repopulation of functioning insulin-producing beta cells in the pancreas.

**Current approaches to prevent type II diabetes include:**

• Increasing insulin sensitivity with adult stem cells:

The insulin receptor signaling pathway is very sensitive to inflammatory mediators.

U.S. FDA-approved Phase III clinical trials are currently being performed for the treatment of graft-versus-host-disease and Crohn's disease by the company Osiris Therapeutics.

• Restoration of insulin production by adult stem cells:

Adult stem cells such as bone marrow and cord blood stem cells, which have been administered to thousands of patients without adverse effects, are already recognized as being capable of differentiating into therapeutic cells such as insulin producing cells. The production of insulin has already been demonstrated in animal models in which mesenchymal stem cells were administered into mice whose beta cells had been damaged by the administration of the toxic compound streptozoicin, after which, increased insulin production was measured in the mice as a direct result of the mesenchymal stem cells.

The use of adult stem cells to induce islet regeneration is also currently undergoing U.S. FDA approved clinical trials at the University of Miami. Additionally, results from numerous clinical studies involving the administration of bone marrow stem cells by physicians outside of the U.S. have been very promising. For example, one group in Argentina has reported that 85% of Type II diabetic patients who were treated with their own mesenchymal stem cells were able to stop using insulin.

**CLINICAL STUDIES:**

There have been 91 diabetic stem cell clinical studies worldwide until October 2011

## III Stem Cell Applications

## III B Key Applications – Dentistry

The regenerative potential of adult stem cells obtained from various sources including dental tissues has been of interest for clinicians over the past years and most research is directed toward achieving the following:

- Regeneration of damaged coronal dentin and pulp
- Regeneration of resorbed root, cervical or apical dentin, and repair perforations
- Periodontal regeneration
- Repair and replacement of bone in craniofacial defects
- Whole tooth regeneration.

**Regeneration of damaged coronal dentin and pulp**

To this date, no restorative material has been able to mimic all physical and mechanical properties of tooth tissue. Furthermore, we have not been successful in providing an ideal solution to certain situations, such as an immature tooth with extensive coronal destruction and reversible pulpitis.

A landmark study conducted by Gronthos *et al*. demonstrated both *in vitro* and *in vivo* in animals that dental pulp stem cells (DPSCs) were capable of forming ectopic dentin and associated pulp tissue. Batouli *et al*. used an *in vivo* stem cell transplantation system to investigate differential regulation mechanisms of bone marrow stromal stem cells (BMSCs) and DPSCs. DPSCs were found to be able to generate a reparative dentin-like tissue on the surface of human dentin *in vivo*.

**Periodontal regeneration**

Regenerating the periodontium has always been a high priority in craniofacial regenerative biology. Due to the complex structure of the periodontium (consisting of hard and soft tissues), its complete regeneration has always remained a challenge. All the current regenerative techniques such as autologous bone grafts, allografts, or alloplastic materials have limitations and cannot be used in all clinical situations. Therefore, a cell-mediated bone regeneration technique will be a viable therapeutic alternative. Kawaguchi *et al*. demonstrated that the transplantation of *ex vivo* expanded autologous MSCs can regenerate new cementum, alveolar bone, and periodontal ligament in class III periodontal defects in dogs. Going a step further, periodontal ligament cells cultured *in vitro* were successfully reimplanted into periodontal defects in order to promote periodontal regeneration by Hasegawa *et al*. A

subsequent study by the same group reported a similar approach in humans. This study reported firm evidence that stem cells can be used to regenerate a tissue as complex as the periodontium.

**Repair and regeneration of bone in craniofacial defects**

Craniofacial bone grafting procedures rely on autologous bone grafting, devitalized allogenic bone grafting (using bone from bone bank), and natural/synthetic osteo-conductive biomaterials. Autologous bone grafting is limited by donor site morbidity and allogenic bone is often destroyed soon. A long-term outcome using biomaterials relies on their ability to encourage local cells to completely regenerate a defect and results are often not encouraging. If stem cells can be harvested in a scaffold and transplanted into a defect to regenerate the lost tissue, it can alleviate a lot of complications associated with the traditional techniques. Abukawa *et al*. used a novel scaffold design with a new fabrication protocol to generate an autologous tissue engineered construct which was used to repair a segmental mandibular defect. In a dog model, Yamada *et al*. showed that a mixture of MSCs and platelet-rich plasma improved bone implant contact and bone density in a mandibular defect. In summary, cell-derived therapy for the repair of osseous defects has been relatively successful and numerous clinical trials in human craniofacial defects are underway.

**Whole tooth regeneration**

A therapeutic option that was unthinkable a few years ago seems an achievable goal today. Even to this day, the replacement of missing teeth has limitations. Although, implants are a significant improvement over dentures and bridges, their fundamental limitation is the lack of natural structural relationship with the alveolar bone (absence of periodontal ligament). They rely on direct integration of bone on tooth surface which is indeed an unnatural relationship as compared with the natural tooth. Further, they are also associated with a lot of esthetic, functional, and surgical limitations that affect their prognosis. Ohazama *et al*. reported the reconstruction of murine teeth using cultured stem cells which when transferred into renal capsules resulted in the development of tooth structures and associated bone. Nakao *et al*. recently engineered teeth ectopically and transplanted them into an anthrotopic site in a mouse jaw. Sonayama *et al*. used SCAP and PDLSCs and formed a bio-root in mini pigs. SCAP and PDLSCs were seeded in a scaffold and implanted into the sockets of the lower jaw. Post channels were pre-created to leave space for post-insertion and 3 months later the bio-root was exposed and a porcelain crown was inserted. The bio-root developed, and had a natural relationship with the surrounding bone.

## III Stem Cell Applications

## III B Key Applications – Parkinson's disease

Parkinson's disease (also known as Parkinson disease, Parkinson's, idiopathic Parkinsonism, primary Parkinsonism, PD, or paralysis agitans) is a degenerative disorder of the central nervous system. It results from the death of dopamine-containing cells in the substantia nigra, a region of the midbrain; the cause of cell-death is unknown. Early in the course of the disease, the most obvious symptoms are movement-related, including shaking, rigidity, slowness of movement and difficulty with walking and gait. Later, cognitive and behavioural problems may arise, with dementia commonly occurring in the advanced stages of the disease. Other symptoms include sensory, sleep and emotional problems. PD is more common in the elderly with most cases occurring after the age of 50.

**Causes:**

Most people with Parkinson's disease have idiopathic Parkinson's disease (having no specific known cause). A small proportion of cases, however, can be attributed to Oxidative stress ,Genetic predisposition ,Exposure to environmental toxins ,Accelerated aging.

**Treatment of Parkinson's disease**:

There is no cure for Parkinson's disease, but medications, surgery and multidisciplinary management can provide relief from the symptoms.

*Drugs:*

Levodopa,Dopamine agonists(bromocriptine, pergolide, pramipexole, ropinirole, piribedil, cabergoline, apomorphine and lisuride),MAO-B inhibitors and other drugs such as amantadine and anticholinergics may be useful as treatment of motor symptoms

*Surgery and deep brain stimulation:*

Placement of an electrode into the brain - The head is stabilized in a frame for stereotactic surgery. Treating motor symptoms with surgery was once a common practice, but since the discovery of levodopa, the number of operations declined.

*Gene therapy:*

Gene therapy involves the use of a non-infectious virus to shuttle a gene into a part of the brain. The gene used leads to the production of an enzyme that helps to manage PD symptoms or protects the brain from further damage.In 2010 there were four clinical trials using gene therapy in PD. There have not been important adverse effects in these trials although the clinical usefulness of gene therapy is still unknown.

*Neuroprotective treatments:*

While several chemical compounds such as GDNF (chemical structure pictured) have been proposed as neuro-protectors in PD, none have proven efficacy. Investigations on neuroprotection are at the forefront of PD research.

**Neural transplantation (STEM CELL TRANSPLANTS)**

Since early in the 1980s, fetal, porcine, carotid or retinal tissues have been used in cell transplants, in which dissociated cells are injected into the substantia nigra in the hope that they will incorporate themselves into the brain in a way that replaces the dopamine-producing cells that have been lost. Stem cell transplants are a recent research target, because stem cells are easy to manipulate and stem cells transplanted into the brains of rodents and monkeys have been found to survive and reduce behavioral abnormalities.

There are two principally different ways of using stem cells for grafting in PD. First, the cells are predifferentiated in vitro to dopaminergic neurons prior to transplantation. Thus, stem cells could become an almost unlimited source for the generation of dopamine neurons. Second, the progenitors differentiate in vivo to dopaminergic neurons after implantation into the striatum or substantia nigra. These neurons may integrate better compared with primary embryonic neurons and, in the ideal scenario, reconstruct the nigrostriatal pathway. Some support for this strategy was provided in a recent report in which investigators found that undifferentiated mouse embryonic stem cells, implanted in low numbers into the dopamine denervated rat striatum, proliferated and that a proportion of them differentiated into cells expressing several markers of mesencephalic dopaminergic neurons.

*In India:*

All India Institute of Medical Sciences (AIIMS) has recently started stem cell trials for the treatment of Parkinson's disease and the neurology department has enrolled five patients for it. Though in the nascent stages, experts say stem cell treatment is likely to be the preferred treatment mode for neurological disorders like Parkinson's, Alzheimer's and the lot in the near future. Stem cell therapy in fact was the recurrent theme in the 2nd Asian and Oceanian Parkinson's Disease and Movement Disorder Congress and the 7th Asia Pacific Parkinson's Association (APPA) organised by AIIMS neurology department.

Usually extracted from embryos or from bone marrow or umbilical cord, stem cells are primitive, undifferentiated cells that have the ability to grow into any type of tissue. They are being hailed as the dream treatment for a wide range of "incurable'' diseases.“We have started stem cell trials for the treatment of Parkinson's disease. But it is a long process. In this trial, we will be using bone marrow stem cells harvested from patients. The cells, after being regrown in the laboratory, will be surgically inserted into the patient's brain. As we would be using patient's own stem cells, there would be no chances of rejection," said Dr Sumit Singh, associate professor and co-investigator of the stem cell trail at AIIMS. Headed by Dr Madhuri Behari, head of the neurology department, AIIMS, the team stated the trial in January. "We plan to inject the stem cells back into the patients in the coming month. But it is too early to predict the results said Dr. Singh.

After Alzheimer's disease, Parkinson's is the second most common neuro-degenerative disorder. It is estimated that worldwide 1% people above the age of 65 and 3% people above the age of 80 are affected by it. In India alone, there are approximately 60 million people suffering from Parkinson's disease. "The reason for the disease is not known, but with right and timely medical intervention, the process of degeneration can be delayed. But factors like genetic composition and environmental toxins are found to be responsible for the disease," said Dr Behari.

Deliberating on the new trends in the treatment of Parkinson's disease, experts cautioned that "hype should be kept aware from hope" in case of stem cell treatment, as trials are in progress. Dr Rupam Borgohain, neurologist at Nizam's Institute of Medical Sciences in Hyderabad, who is also experimenting with stem cells in treating Parkinson's disease, said, "It (stem cells) is definitely the way to go, but it is important to keep hype away from hope. It is in the trial phase and we hope to see positive results, but till then we have to wait. Stem cell tissues have proved to be beneficial in treating Parkinson's disease in a few cases. And that gives us hope to move forward."

## III Stem Cell Applications

## III D Key Applications – Myocardial Infarction

**Myocardial infarction** (**MI**) or **acute myocardial infarction** (**AMI**), commonly known as a **heart attack**, results from the interruption of blood supply to a part of the heart, causing heart cells to die

**Causes, incidence, and risk factors:**

Most heart attacks are caused by a blood clot that blocks one of the coronary arteries. The coronary arteries bring blood and oxygen to the heart. If the blood flow is blocked, the heart is starved of oxygen and heart cells die. A hard substance called plaque can build up in the walls of your coronary arteries. This plaque is made up of cholesterol and other cells. A heart attack can occur as a result of plaque buildup.

- The plaque can develop cracks or tears. Blood platelets stick to these tears and form a blood clot. A heart attack can occur if this blood clot completely blocks oxygen-rich blood from flowing to the heart. This is the most common cause of heart attacks.

- The slow buildup of plaque may almost block one of your coronary arteries. A heart attack may occur if not enough oxygen-rich blood can flow through this blockage. This is more likely to happen when your body is stressed (for example, by a serious illness).

**Symptoms**

Chest pain is the most common symptom of a heart attack. The pain is felt in only one part of the body, or it may move from chest to your arms, shoulder, neck, teeth, jaw, belly area, or back.

**Treatment**

Abnormal heartbeats (arrhythmias) are the leading cause of death in the first few hours of a heart attack. These arrhythmias may be treated with medications or cardio version.

EMERGENCY TREATMENTS

Angioplasty is a procedure to open narrowed or blocked blood vessels that supply blood to the heart. Usually a small, metal mesh tube called a stent is placed at the same time.

- Angioplasty is often the first choice of treatment. It should be done within 90 minutes after you get to the hospital, and no later than 12 hours after a heart attack.
- A stent is a small, metal mesh tube that opens up (expands) inside a coronary artery. A stent is often placed after angioplasty. It helps prevent the artery from closing up again.

Sometimes drugs are given to break up the clot within 3 hours of the chest pain. This is called thrombolytic therapy. Some patients may also have heart bypass surgery to open narrowed or blocked blood vessels that supply blood to the heart. This procedure is also called open heart surgery.

The following drugs are given to most people after they have a heart attack. These drugs can help prevent another heart attack. Antiplatelet drugs (blood thinners) such as aspirin, clopidogrel (Plavix), or warfarin (Coumadin), to help keep your blood from clotting

- Beta-blockers and ACE inhibitor medicines to help protect your heart
- Statins or other drugs to improve your cholesterol levels

Stem cell treatment:

How might stem cells play a part in repairing the heart? To answer this question, researchers are building their knowledge base about how stem cells are directed to become specialized cells. One important type of cell that can be developed is the cardiomyocyte, the heart muscle cell that contracts to eject the blood out of the heart's main pumping chamber (the ventricle). Two other cell types are important to a properly functioning heart are the vascular endothelial cell, which forms the inner lining of new blood vessels, and the smooth muscle cell, which forms the wall of blood vessels. The heart has a large demand for blood flow, and these specialized cells are important for developing a new network of arteries to bring nutrients and oxygen to the cardiomyocytes after a heart has been damaged. The potential capability of both embryonic and adult stem cells to develop into these cells types in the damaged heart is now being explored as part of a strategy to restore heart function to people who have had heart attacks or have congestive heart failure.

Researchers now know that under highly specific growth conditions in laboratory culture dishes, stem cells can be coaxed into developing as new cardiomyocytes and vascular endothelial cells. Scientists are interested in exploiting this ability to provide replacement tissue for the damaged heart. This approach has immense advantages over heart transplant, particularly in light of the paucity of donor hearts available to meet current transplantation needs.

Recent developments in stem-cell research suggest the possibility of repairing damage. Stem cells are undeveloped cells that have the potential to develop into any type of specialized cell in the body,

including heart muscle cells (cardiomyocytes). Transplanted stem cells seem to be able to attach themselves to the site of injury and replace cells that have been lost..
Replacing damaged heart muscle cells with healthy cells may avoid heart failure after myocardial infarction.

**Stem Cell Therapy in MI**

**Study – I**

**Title –** Intracoronary bone marrow cell transfer after myocardial infarction: 5-year follow-up from the randomised-controlled BOOST trial.

**Researchers –** Gerd P. Meyer et al.

**Organisation –** Hannover Medical School, Germany

**Year of study –** 2009

**Aims –** To assess whether a single intracoronary infusion of autologous bone marrow cells (BMCs) can have a sustained impact on left ventricular ejection fraction (LVEF) in patients after ST-elevation myocardial infarction (STEMI).

**Sample size –** 60

**Conclusion –** A single intracoronary application of BMCs does not promote a sustained improvement of LVEF in STEMI patients with relatively preserved systolic function. It is conceivable that a subgroup of patients with more transmural infarcts may derive a sustained benefit from BMC therapy. However, this needs to be tested prospectively in a randomised trial.

**Study – II**

**Title –** The acute and long-term effects of intracoronary stem cell transplantation in 191 patients with chronic heart failure: the STAR-heart study

**Researchers –** B.-E. Strauer et al

**Organisation –** Heinrich-Heine-University of Dusseldorf, Germany

**Year of Study –** 2010

**Aims –** To quantitatively investigate ventricular haemodynamics, geometry, and contractility as well as the long-term clinical outcome of BMC treated patients with reduced left ventricular ejection fraction (LVEF) due to chronic ischaemic cardiomyopathy.

**Sample size –** 391

**Conclusion –** Intracoronary BMC therapy improves ventricular performance, quality of life and survival in patients with heart failure. These effects were present when BMC were administered in addition to standard therapeutic regimes. No side effects were observed.

**Study – III**

**Title –** Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial

**Researchers –** V. Schachinger et al

**Organisations –** 1 J. W. Goethe Universita¨t Frankfurt, Med. Klinik III, Abt. Kardiologie, Theodor-Stern-Kai 7, 60590 Frankfurt a. M., Germany; et all

**Year of study –** 2006

**Aims –** To investigate the clinical outcome after intracoronary administration of autologous progenitor cells in patients with acute myocardial infarction (AMI)

**Sample size –** 204

**Conclusion –** Intracoronary administration of BMCs is associated with a significant reduction of the occurrence of major adverse cardiovascular events after AMI. Large-scale studies are warranted to confirm the effects of BMC administration on mortality and morbidity in patients with AMIs.

# IV Stem Cell Banking

## IV A Basics of Stem cell Banking

A stem cell bank is a facility which stores stem cells, either in the isolated form or in the source tissue form (processed cord tissue, adipose tissue, dental pulp, etc) for future use. Stem cell bank can be of various types based on the source of stem cells; viz. dental pulp stem cell bank stores dental pulp containing the stem cells; cord blood bank stores umbilical cord blood; etc.

Stem cell banks are also classified as Public or Private based on the donations.

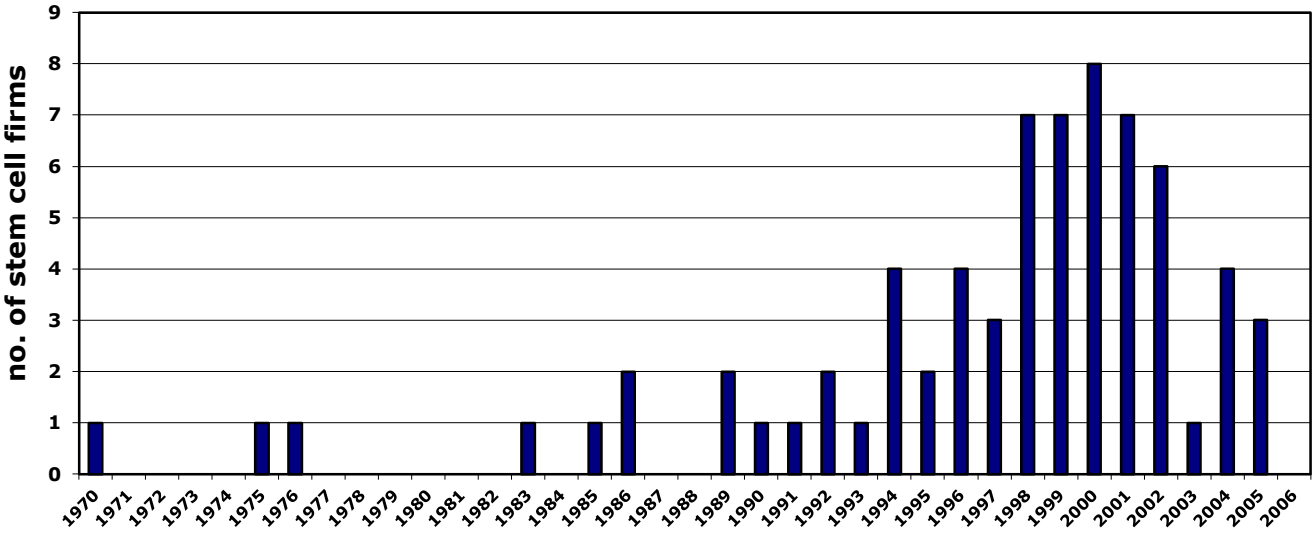
*Public bank*

Public banks accept donations to be used for anyone in need. Unlike private banking, public banking is supported by the medical community. However, there are very strict regulations which public banks need to follow in order to enable the donated units to be added to a registry. In case of cord blood public banking (which are present in India as well as abroad), an expectant mother interested in donation should contact the bank before the 34th week of pregnancy. Once the blood is donated, it loses all identifying information after a short period of initial testing. Families are not able to retrieve their own blood after it has been donated.

*Private bank*

Banking stem cells in private banks is a personal choice made by parents (in case of minor) or the adult (personal donation). Private Banks store stem cells or stem cell tissue with a link to the identity of the donor, so that the family may retrieve it later if it is needed. The parents have custody of the stem cells/stem cell tissue until the child is an adult. The stem cell/stem cell tissue might someday be needed by the donor baby, or it could be used by a relative who is a close enough match to receive a transplant from the donor (typically a sibling). Stemade is a private bank, so is Lifecell. Reliance has both the facilities for the cord blood.

Stem Cell banks are growing in number with many foreign collaborators tying up with Indian companies to set out private / public banks. Global Scenario - The concept of treatment using stem cells has evolved in the last 10-15 years; Market size for stem cell therapy in 2006 was estimated at $26 billion and is expected to touch the $96 billion level by 2015




Indian Scenario - In India umbilical cord / tissue banks have flourished over the last decade. Prominent stem cell banks in India are Lifecell International Pvt. Ltd (Chennai), Cryobanks (Delhi), Reliance Life Sciences (Mumbai) and Cryo Save (Banglore). Lifecell also banks menstrual blood stem cells.

Banking Procedures:

Any stem cell bank/stem cell tissue bank involves primarily 3 procedures:

- Collection
- Isolation
- Cryopreservation

The detailed procedure adopted by Stemade for dental pulp stem cell banking is explained in the following chapter.

*Advantages of banking*

Stem cells are currently used in the treatment of several life-threatening diseases, and play an important role in the treatment of blood/ connective tissue disorders related to neuropsychiatry, cancers, cardiac, etc.

In certain instances, there may be some medical issues around using one's own stem cells, as well as availability of cells, which will require treatments done using cells from another donor, with the vast

majority being unrelated donors. However, studies have shown that stem cells can also be used for siblings and other members of your family who have a matching tissue type. Siblings have up to a 75% chance of compatibility, and the stem cells may even be a match for parents (50%) and grandparents.

# IV Stem Cell Banking

## IV B Essentials of Transplantation

*Stem Cell Transplantation*

Organ transplantation is the moving of an organ from one body to another or from a donor site on the patient's own body, for the purpose of replacing the recipient's damaged or absent organ. The emerging field of regenerative medicine is allowing scientists and engineers to create organs to be re-grown from the patient's own cells (stem cells, or cells extracted from the failing organs). Organs and/or tissues and/or stem cells that are transplanted within the same person's body are called autografts. Transplants that are performed between two subjects of the same species are called allografts. Allografts can either be from a living or cadaveric source.

- Autotransplantation (autologous transplants)

Autotransplantation is the transplantation of cells, tissues or organs or even proteins from one part of the body to another in the same individual. Tissue transplanted by such "autologous" procedure is referred to as an autograft or autotransplant. Sometimes this is done with surplus tissue, or tissue that can regenerate, or tissues more desperately needed elsewhere (examples include skin grafts, etc.) Sometimes an autograft is done to remove the tissue and then treat it or the person, before returning it (examples include stem cell autograft and storing blood in advance of surgery).

- Allotransplantation (Allogenic transplants)

Allotransplantation is the transplantation of cells, tissues or organs, sourced from a genetically non-identical member of the same species as the recipient. The transplant is called an allograft or allogeneic transplant or homograft. Most human tissue and organ transplants are allografts. In contrast, a transplant from another species is called a xenograft. A transplanted organ or tissue from a genetically identical donor, i.e., an identical twin, is termed an isograft.
When a host mounts an immune response against an allograft or xenograft, the process is termed rejection. An allogenic bone marrow transplant can result in an immune attack against recipient, termed Graft-versus-host disease.

- Graft- versus-host disease

Graft-versus-host disease (GVHD) is a common complication after a stem cell transplant or bone marrow transplant from another person (an allogenic transplant). Immune cells (white blood cells) in the donated marrow or stem cells (the graft) recognize the recipient (the host) as "foreign". The transplanted immune cells then attack the host's body cells. Graft versus host disease can also take place during a blood transfusion under certain circumstances.

- Prevention
- *HLA Matching*

Matching of stem cell donor to a recipient is determined by comparing their tissue types, also known as their Human Leucocyte Antigen (HLA) types. An individual's HLA type is present on nearly all tissues in the body. The white cells from a blood sample are a convenient source of "tissue" that the laboratory can use to determine an individual's HLA type.

HLA typing is important since the degree of HLA compatibility between donor and recipient will influence the outcome of the transplant. The function of the immune system is to fight against foreign particles that the body sees as "non-self", such as bacteria and viruses. A stem cell transplant from an HLA mismatched donor can result in the recipient's immune system recognising the transplanted cells as "non-self" and attacking the cells as it would bacteria or viruses. This can lead to rejection of the transplanted stem cells. Likewise cells from the donor's immune system which are introduced along with the transplanted stem cells ("graft") can also recognise HLA mismatches and attack vital organs of the recipient's body ("host"). This is called graft versus host disease (GvHD). The more compatible the donor-recipient match, the less likely it is that rejection or severe GvHD will occur.

A 20-30 ml blood sample is required to perform HLA typing. The white cells are isolated from the blood and typing is performed by two different methods:

- Serological testing: where the white cells are used
- DNA testing: where DNA extracted from the white cells is used.

When DNA testing is performed there is no breach of a person’s confidentiality. The DNA is not used for any reasons other than tissue typing and ethically approved research purposes, and remains the property of the tissue typing laboratory.
Preliminary tissue typing takes about 2 weeks. Further high resolution (more detailed) tissue typing performed on the patient and any potentially matched donor samples may take another 2 to 4 weeks.

HLA typing is reported as a series of numbers. For example, an HLA type will appear on the report as $A_{3,32}$; $B_{7,37}$; $DR_{1,15}$. Results of family members and/or unrelated donors are compiled with the patient’s

HLA typing results and reported directly to the clinician by the laboratory that performed the tests. The clinician will inform the patient and family of matches found and will be available to discuss the results.

An HLA type consists of two main groups: Class I antigens (HLA-A, -B, -C) and Class II antigens (HLA-DR, -DQ, -DP). There are six HLA antigens considered most important for determining compatibility: two A antigens, two B antigens, and two DR antigens (eg: $A_{3,\ 32}$; $B_{7,\ 37}$; $DR_{1,15}$). We inherit a set (or haplotype) of HLA-A, B and DR antigens from each parent.

The two inherited haplotypes determine the HLA type of an individual consisting of two HLA-A antigens, two HLAB antigens and two HLA-DR antigens. Since the maternal and paternal haplotypes can combine in four different ways (as shown in the figure above), there are four possible HLA types that can be inherited by the children. This means that statistically each child has a one in four chance of being HLA matched with any one sibling. HLA matching is not related to appearance or personality between family members, blood group or sex.

**Dental stem cell bank**

Dental stem cell bank is a facility that stores stem cells derived from teeth for future use. The concept can be compared to a blood bank where blood is stored for future use. Likewise, stem cell samples are stored specifically for the use by the individual person from whom such cells have been collected. The sample can be later retrieved by the individual for therapeutic purposes

**Annexure I - Glossary**

Adult stem cell—See *Somatic stem cell.*

Astrocyte—A type of supporting (glial) cell found in the nervous system.

Blastocoel—The fluid-filled cavity inside the blastocyst, an early, preimplantation stage of the developing embryo.

Blastocyst—A preimplantation embryo of about 150 cells produced by cell division following fertilization. The blastocyst is a sphere made up of an outer layer of cells (the trophoblast), a fluid-filled cavity (the blastocoel), and a cluster of cells on the interior (the inner cell mass).

Bone marrow stromal cells—A population of cells found in bone marrow that are different from blood cells.

Bone marrow stromal stem cells (skeletal stem cells)—A multipotent subset of bone marrow stromal cells able to form bone, cartilage, stromal cells that support blood formation, fat, and fibrous tissue.

Cell-based therapies—Treatment in which stem cells are induced to differentiate into the specific cell type required to repair damaged or destroyed cells or tissues.

Cell culture—Growth of cells *in vitro* in an artificial medium for experimental research.

Cell division—Method by which a single cell divides to create two cells. There are two main types of cell division depending on what happens to the chromosomes: mitosis and meiosis.

Chromosome—A structure consisting of DNA and regulatory proteins found in the nucleus of the cell. The DNA in the nucleus is usually divided up among several chromosomes. The number of chromosomes in the nucleus varies depending on the species of the organism. Humans have 46 chromosomes.

Clone—(v) To generate identical copies of a region of a DNA molecule or to generate genetically identical copies of a cell, or organism; (n) The identical molecule, cell, or organism that results from the cloning process.

1. In reference to DNA: To clone a gene, one finds the region where the gene resides on the DNA and copies that section of the DNA using laboratory techniques.
2. In reference to cells grown in a tissue culture dish:a clone is a line of cells that is genetically identical to the originating cell. This cloned line is produced by cell division (mitosis) of the original cell.
3. In reference or organisms: Many natural clones are produced by plants and (mostly invertebrate) animals. The term clone may also be used to refer to an animal produced by somatic cell nuclear transfer (SCNT) or parthenogenesis.

Cloning—See *Clone*.

Cord blood stem cells—See *Umbilical cord blood stem cells.*

Culture medium—The liquid that covers cells in a culture dish and contains nutrients to nourish and support the cells. Culture medium may also include growth factors added to produce desired changes in the cells.

Differentiation—The process whereby an unspecialized embryonic cell acquires the features of a specialized cell such as a heart, liver, or muscle cell. Differentiation is controlled by the interaction of a cell's genes with the physical and chemical conditions outside the cell, usually through signaling pathways involving proteins embedded in the cell surface.

Directed differentiation—The manipulation of stem cell culture conditions to induce differentiation into a particular cell type.

DNA—Deoxyribonucleic acid, a chemical found primarily in the nucleus of cells. DNA carries the instructions or blueprint for making all the structures and materials the body needs to function. DNA consists of both genes and non-gene DNA in between the genes.

Ectoderm—The outermost germ layer of cells derived from the inner cell mass of the blastocyst; gives rise to the nervous system, sensory organs, skin, and related structures.

Embryo—In humans, the developing organism from the time of fertilization until the end of the eighth week of gestation, when it is called a fetus.

Embryoid bodies—Rounded collections of cells that arise when embryonic stem cells are cultured in suspension. Embryoid bodies contain cell types derived from all 3 germ layers.

Embryonic germ cells—Pluripotent stem cells that are derived from early germ cells (those that would become sperm and eggs). Embryonic germ cells (EG cells) are thought to have properties similar to embryonic stem cells.

Embryonic stem cells—Primitive (undifferentiated) cells derived from a 5-day preimplantation embryo that are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers.

Embryonic stem cell line—Embryonic stem cells, which have been cultured under *in vitro* conditions that allow proliferation without differentiation for months to years.

Endoderm—The innermost layer of the cells derived from the inner cell mass of the blastocyst; it gives rise to lungs, other respiratory structures, and digestive organs, or generally "the gut".

Enucleated—Having had its nucleus removed.

Epigenetic—Having to do with the process by which regulatory proteins can turn genes on or off in a way that can be passed on during cell division.

Feeder layer—Cells used in co-culture to maintain pluripotent stem cells. For human embryonic stem cell culture, typical feeder layers include mouse embryonic fibroblasts (MEFs) or human embryonic fibroblasts that have been treated to prevent them from dividing.

Fertilization—The joining of the male gamete (sperm) and the female gamete (egg).

Fetus—In humans, the developing human from approximately eight weeks after conception until the time of its birth.

Gamete—An egg (in the female) or sperm (in the male) cell. See also *Somatic cell.*

Gastrulation—The process in which cells proliferate and migrate within the embryo to transform the inner cell mass of the blastocyst stage into an embryo containing all three primary germ layers.

Gene—A functional unit of heredity that is a segment of DNA found on chromosomes in the nucleus of a cell. Genes direct the formation of an enzyme or other protein.

Germ layers—After the blastocyst stage of embryonic development, the inner cell mass of the blastocyst goes through gastrulation, a period when the inner cell mass becomes organized into three distinct cell layers, called germ layers. The three layers are the ectoderm, the mesoderm, and the endoderm.

Hematopoietic stem cell—A stem cell that gives rise to all red and white blood cells and platelets.

Human embryonic stem cell (hESC)— A type of pluripotent stem cell derived from early-stage human embryos, up to and including the blastocyst stage. hESCs are capable of dividing without differentiating for a prolonged period in culture and are known to develop into cells and tissues of the three primary germ layers. See also *pluripotent, blastocyst,* and *germ layers.*

Induced pluripotent stem cell (iPSC)— A type of pluripotent stem cell, similar to an embryonic stem cell, formed by the introduction of certain embryonic genes into a somatic cell.

*In vitro*—Latin for "in glass"; in a laboratory dish or test tube; an artificial environment.

*In vitro* fertilization—A technique that unites the egg and sperm in a laboratory, instead of inside the female body.

Inner cell mass (ICM)—The cluster of cells inside the blastocyst. These cells give rise to the embryo and ultimately the fetus. The ICM cells may be used to generate embryonic stem cells.

Long-term self-renewal—The ability of stem cells to renew themselves by dividing into the same non-specialized cell type over long periods (many months to years) depending on the specific type of stem cell.

Mesenchymal stem cells—A term that is currently used to define non-blood adult stem cells from a variety of tissues, although it is not clear that mesenchymal stem cells from different tissues are the same.

Meiosis—The type of cell division a diploid germ cell undergoes to produce gametes (sperm or eggs) that will carry half the normal chromosome number. This is to ensure that when fertilization occurs, the fertilized egg will carry the normal number of chromosomes rather than causing aneuploidy (an abnormal number of chromosomes).

Mesoderm—Middle layer of a group of cells derived from the inner cell mass of the blastocyst; it gives rise to bone, muscle, connective tissue, kidneys, and related structures.

Microenvironment—The molecules and compounds such as nutrients and growth factors in the fluid surrounding a cell in an organism or in the laboratory, which play an important role in determining the characteristics of the cell.

Mitosis—The type of cell division that allows a population of cells to increase its numbers or to maintain its numbers. The number of chromosomes remains the same in this type of cell division.

Multipotent—Having the ability to develop into more than one cell type of the body. See also *pluripotent* and *totipotent*.

Neural stem cell—A stem cell found in adult neural tissue that can give rise to neurons and glial (supporting) cells. Examples of glial cells include astrocytes and oligodendrocytes.

Neurons—Nerve cells, the principal functional units of the nervous system. A neuron consists of a cell body and its processes—an axon and one or more dendrites. Neurons transmit information to other neurons or cells by releasing neurotransmitters at synapses.

Oligodendrocyte—A supporting cell that provides insulation to nerve cells by forming a myelin sheath (a fatty layer) around axons.

Parthenogenesis—The artificial activation of an egg in the absence of a sperm; the egg begins to divide as if it has been fertilized.

Passage—In cell culture, the process in which cells are disassociated, washed, and seeded into new culture vessels after a round of cell growth and proliferation. The number of passages a line of cultured cells has gone through is an indication of its age and expected stability.

Pluripotent—Having the ability to give rise to all of the various cell types of the body. Pluripotent cells cannot make extra-embryonic tissues such as the amnion, chorion, and other components of the placenta. Scientists demonstrate pluripotency by providing evidence of stable developmental

potential, even after prolonged culture, to form derivatives of all three embryonic germ layers from the progeny of a single cell and to generate a teratoma after injection into an immunosuppressed mouse.

Polar Body—A polar body is a structure produced when an early egg cell, or oogonium, undergoes meiosis. In the first meiosis, the oogonium divides its chromosomes evenly between the two cells but divides its cytoplasm unequally. One cell retains most of the cytoplasm, while the other gets almost none, leaving it very small. This smaller cell is called the first polar body. The first polar body usually degenerates. The ovum, or larger cell, then divides again, producing a second polar body with half the amount of chromosomes but almost no cytoplasm. The second polar body splits off and remains adjacent to the large cell, or oocyte, until it (the second polar body) degenerates. Only one large functional oocyte, or egg, is produced at the end of meiosis.

Preimplantation—With regard to an embryo, preimplantation means that the embryo has not yet implanted in the wall of the uterus. Human embryonic stem cells are derived from preimplantation stage embryos fertilized outside a woman's body *(in vitro).*

Proliferation—Expansion of the number of cells by the continuous division of single cells into two identical daughter cells.

Regenerative medicine—A field of medicine devoted to treatments in which stem cells are induced to differentiate into the specific cell type required to repair damaged or destroyed cell populations or tissues. See also *cell-based therapies*.

Reproductive cloning—The process of using somatic cell nuclear transfer (SCNT) to produce a normal, full grown organism (e.g., animal) genetically identical to the organism (animal) that donated the somatic cell nucleus. In mammals, this would require implanting the resulting embryo in a uterus where it would undergo normal development to become a live independent being. The first animal to be created by reproductive cloning was Dolly the sheep, born at the Roslin Institute in Scotland in 1996. See also *Somatic cell nuclear transfer (SCNT).*

Signals—Internal and external factors that control changes in cell structure and function. They can be chemical or physical in nature.

Somatic cell—Any body cell other than gametes (egg or sperm); sometimes referred to as "adult" cells. See also *Gamete.*

Somatic cell nuclear transfer (SCNT)—A technique that combines an enucleated egg and the nucleus of a somatic cell to make an embryo. SCNT can be used for therapeutic or reproductive purposes, but the initial stage that combines an enucleated egg and a somatic cell nucleus is the same. See also *Therapeutic cloning* and *Reproductive cloning.*

Somatic (adult) stem cell—A relatively rare undifferentiated cell found in many organs and differentiated tissues with a limited capacity for both self renewal (in the laboratory) and

differentiation. Such cells vary in their differentiation capacity, but it is usually limited to cell types in the organ of origin. This is an active area of investigation.

Stem cells—Cells with the ability to divide for indefinite periods in culture and to give rise to specialized cells.

Stromal cells—Connective tissue cells found in virtually every organ. In bone marrow, stromal cells support blood formation.

Subculturing—Transferring cultured cells, with or without dilution, from one culture vessel to another.

Surface markers—Proteins on the outside surface of a cell that are unique to certain cell types and that can be visualized using antibodies or other detection methods.

Teratoma—A multi-layered benign tumor that grows from pluripotent cells injected into mice with a dysfunctional immune system. Scientists test whether they have established a human embryonic stem cell (hESC) line by injecting putative stem cells into such mice and verifying that the resulting teratomas contain cells derived from all three embryonic germ layers.

Therapeutic cloning—The process of using somatic cell nuclear transfer (SCNT) to produce cells that exactly match a patient. By combining a patient's somatic cell nucleus and an enucleated egg, a scientist may harvest embryonic stem cells from the resulting embryo that can be used to generate tissues that match a patient's body. This means the tissues created are unlikely to be rejected by the patient's immune system. See also *Somatic cell nuclear transfer (SCNT).*

Totipotent—Having the ability to give rise to all the cell types of the body plus all of the cell types that make up the extraembryonic tissues such as the placenta. See also *Pluripotent* and *Multipotent*.

Transdifferentiation—The process by which stem cells from one tissue differentiate into cells of another tissue.

Trophectoderm—The outer layer of the preimplantation embryo in mice. It contains trophoblast cells.

Trophoblast—The outer cell layer of the blastocyst. It is responsible for implantation and develops into the extraembryonic tissues, including the placenta, and controls the exchange of oxygen and metabolites between mother and embryo.

Umbilical cord blood stem cells—Stem cells collected from the umbilical cord at birth that can produce all of the blood cells in the body (hematopoietic). Cord blood is currently used to treat patients who have undergone chemotherapy to destroy their bone marrow due to cancer or other blood-related disorders.

Undifferentiated—A cell that has not yet developed into a specialized cell type.

**Annexure II - Chronology of advances in stem cell research**

June 1, 1909: Alexander Maximow presents a lecture at the Hematological Society of Berlin introducing the concept of stem cells as the common ancestors of cellular elements in the blood.

1959: First successful use of stem cell transplants in humans, in three separate studies all involving hematopoietic stem cells (HSCs). E. D. Thomas and colleagues use syngeneic grafts from identical twins to treat two leukemia patients, George Mathé and colleagues perform allogeneic (from a separate individual who is not an identical twin) bone marrow transplants on five patients accidentally exposed to irradiation, and McGovern and colleagues treat a leukemia patient with autologous (from the patient) bone marrow cells.

1963: E. A. McCullough and colleagues prove that stem cells exist in the bone marrow of mice and that HSCs have the key properties of self-renewal and could become any type of blood cell.

June 1966: R. J. Cole, R. G. Edwards, and J. Paul isolate embryonic stem cells (ESCs) from the pre-implantation blastocysts of rabbits.

1968: First successful use of bone marrow transplantation to treat patients with leukemia or hereditary immunodeficiency: success due to presence of HSCs in the marrow graft, which can reconstitute blood and immune systems after myeloablation.

1974: Congress imposes moratorium on federal funding for clinical research on embryonic tissue and embryos, which remains in place until 1993.

1981: *Nature* announces that two research groups, working independently, successfully derived embryonic stem cells from the inner cell mass cells of the blastocyst in mice; one group is led by Martin Evans at the University of Cambridge (UK), the other by Gail Martin at the University of California, San Francisco.

1987: Peter Hollands demonstrates the first therapeutic in vivo (in a living animal) use of ESCs: injection of ESCs restores lost bone marrow stem cells in lethally irradiated mice.

1988: Bone Marrow Donors Worldwide, a collaborative network of stem cell donor registries and cord blood banks, founded in Leiden (the Netherlands) to facilitate sharing of HLA phenotype and other information to physicians of patients who need a hematopoietic stem cell transplant.

1992: Y. Matsui and colleagues announce successful isolation of mouse embryonic germ cells, which have properties similar to embryonic stem cells.

January 1993: Newly elected president Bill Clinton instructs Donna Shalala, Secretary of the U.S. Department of Health and Human Services, to remove the ban on embryonic research.
1995: Congress bans federal funding for research on embryos, but leaves it unclear whether this ban applies to cells already derived from an embryo.

November 1995: James A. Thomson and colleagues at the University of Wisconsin derive the first non-human primate embryonic stem cells, from rhesus monkeys, suggesting that embryonic stem cells could also be derived from humans.

November 5 and 10, 1998: James A. Thomson at the University of Wisconsin, and John D. Gearhart at Johns Hopkins University report almost simultaneously that they have successfully isolated human embryonic stem cells (hESCs). Despite the therapeutic potential of hESCs, which can become any type of cell in the human body and thus offer hope for currently intractable conditions such as Parkinson's disease and spinal cord injury, the announcement is not without controversy due to the origins of the cells used in the research. Thomson's team worked with cells from human embryos created in vitro ("in glass," i.e., in the laboratory) while Gearhart's team obtained their stem cells from human fetal primordial germ cells.

August 2000: The National Institutes of Health (NIH) legal department advices that NIH may fund research on cells derived from blastocysts, but may not fund the derivation of the cells themselves (which may be performed by private companies).

December 2000: Mouse experiments by Timothy Brazelton and colleagues at Stanford University discover that HSCs can transform themselves to neuronal cells, demonstrating a plasticity (ability to become other types of cells than blood cells) which could have important therapeutic implications). This research has been challenged on several grounds but research continues because of the ready availability of HSCs (every person could serve as their own donor, making hESCs unnecessary).

July 2001: The Jones Institute, a private infertility clinic in Norfolk, Virginia, announces that it has created embryos from donated gametes (reproductive cells).

August 9, 2001: President George W. Bush, in a speech on prime-time national television, announces federal research funding will be available for the first time for hESC research, but that such research would be limited to the estimated 60 pre-existing stem cell lines.

November 2001: NIH invites proposals for stem cell research and releases a list of 74 acceptable stem cell lines; many of the lines are not suitable for human trials because they have been grown in mouse media.

November 25, 2001: Advanced Cell Technology, a private company in Worcester, Massachusetts, announces that it has cloned human embryos from adult cells, creating cells that are a perfect genetic match for the donor.

2002: The United Kingdom announces that stem cell research is a scientific priority and allocates an additional £40 million to support stem cell research.

January 2003: Nine funding agencies form the International Stem Cell Forum (ISCF) to encourage international collaboration and promote increased funding for stem cell research; as of January 2004,14 agencies from 13 countries have joined the ISCF.

2004: Annual Report of the International Bone Marrow Transplant Registry reports that over 27,000 patients annually are treated by blood stem cell transplantation, for various cancers, hereditary diseases, and bone marrow failure

March 2004: Hwang Woo-Suk and colleagues at Seoul National University announces in the prestigious journal *Science* that he successfully cloned patent-specific stem cells suing somatic nuclear transfer. Because the embryos were cloned in order to produce stem cells, rather than for reproduction, this reported success reopens the debate about therapeutic cloning (cloning cells for the purpose of treating human disease). Hwang's previous research had been in genetically modified livestock, and he claimed to have successfully cloned two cows in 1999, although he provided no scientific data to back up this claim.

June 25, 2004: New Jersey becomes the first state to fund stem cell research, as legislators create the Stem Cell Institute of New Jersey and allocate it $9.5 million in state funding.

November 2, 2004: Partly as a response to federal research funding restrictions, California becomes the second state to allocate funding for stem cell research, as voters approve Proposition 71. This bill creates the California Institute for Regenerative Medicine, which is allocated $3 billion in taxpayer funding over 10 years.

January 1, 2005: Connecticut Governor M. Jodi Rell announces that she will recommend that the state budget include a special fund to support stem cell research in Connecticut. The state budget, passed in June, includes $100 million to support stem cell research over 10 years.

May 23, 2005: The Starr Foundation announces awards of $50 million to support stem cell research at Weill Medical College of Cornell University, Rockefeller University, and Memorial Sloan-Kettering Cancer, all in New York City.

May 31, 2005: The State of Connecticut Stem Cell Advisory Committee allocates $19.78 million in stem cell research funds to researchers from Yale, Wesleyan, and the University of Connecticut. These are the first grants from Connecticut's Stem Cell Research Fund, which was created in 2005 and is charged with allocating approximately $100 million to support stem cell research by the year 2015.

June 2005: Hwang Woo-Suk and colleagues publish an article in *Science* claiming that they have created 11 human embryos from somatic cells from different donors. He claims to have developed a more efficient process that uses fewer eggs to create more hESCs.

July 13, 2005: Illinois Governor Rod Blagojevich issues an executive order which creates the Illinois Regenerative Institute for Stem Cell Research, which will award $10 million in state funds to support stem cell research. This makes Illinois the fourth state, and the first midwestern state, to allocate public funds to stem cell research.

August 18, 2005: Colin McGuckin, Nico Forraz and colleagues at Kingston University (UK) announce discovery of cord-blood-derived embryonic- like stem cells (CBEs), which appear to be more versatile than adult stem cells found in bone marrow, although less versatile than hESCs. This discovery could skirt ethical objections to hESC research with cells derived from embryos, because umbilical cord blood can be acquired without destruction of human life.

September 19, 2005: Brian Cummings, Aileen Anderson and colleagues at the University of California, Irvine, announce that they successfully used adult neural stem cells to repair spinal cord damage in

mice. The mice receiving neural stem cells showed improvement in coordination and walking ability, suggesting the research may lead to therapies to aid humans with spinal cord injuries.

September 21, 2005: Floridians for Stem Cell Research and Cures, Inc., an advocacy group for stem cell research, propose a ballot initiative requiring the state of Florida to spend $200 million in state funds over the next 10 years in support of stem cell research. On September 23, Citizens for Science and Ethics, Inc., a group opposing stem cell research, files a petition which would amend Florida's state constitution to prohibit embryonic stem cell research.
November 2005: Gerald Schatten a former colleague of Hwang Woo-Suk now at the University of Pennsylvania, announces there were ethical irregularities in Hwang's procurement of oocyte (egg) donations used in his research. Roh Sungil, a close collaborator, announces at a press conference on November 21 that oocyte donors had been paid $1,400 each for their eggs. On November 24, Hwang announces that he will resign from his post due to the scandal.

December 16, 2005: New Jersey becomes the first state to allocate public funds for hESC research, as a state commission grants $5 million awarded to 17 research projects, most located at the University of Medicine and Dentistry of New Jersey, Rutgers University, and Princeton University.

December 29, 2005: In South Korea, a Seoul National University investigation of Hwang's scientific work concludes that all 11 stem cell lines claimed in his 2005 published paper were fabricated.

2006 (calendar year): Over 1,100 articles on ESC research are published, a nearly 10-fold increase from 140 in 1997.

January 11, 2006: *Science* retracts both of Hwang's papers due to scientific misconduct and fraud. On January 12, Hwang holds a press conference to apologize but does not take responsibility for the fraud claiming that members of his scientific team sabotaged his work.

April 2006: Maryland allocates $15 million in state funding for ESC research, beginning in July 2006, through passage of the Stem Cell Research Act.

May 12, 2006: South Korea indicts scientist Hwang Woo-suk on charges of fraud, embezzlement, and bioethics violations. Three of his collaborators are also charged with fraud.

June 21, 2006: Florida Governor Jeb Bush, speaking at the annual biotechnology Industry Organization meeting, announces his disapproval of hESC research. Bush further announces that no stem cell research will be performed at any Florida university, nor at the Scripps Research Institute in Palm Beach.

July 2006: ES Cell International in Singapore becomes the first company to commercially produce hESCs that are suitable for clinical trials; vials of stems cells are offered for sale on the internet for $6,000.

July 18, 2006: Senate Majority Leader Bill Frist (R-TN) publishes an editorial in the *Washington Post* announcing his support of federal funding of stem cell research, in opposition to President Bush's policy. Frist also announces that he sees no contradiction between stem cell research and his pro-life beliefs.

July 19, 2006: President Bush vetoes a bill, passed by the House in 2005 and the Senate in July 2006, that would expand federal funding for hESC research.
August 23, 2006: Scientists from the private company Advanced Cell Technology announce they have developed a technique which allows them to remove a single cell from an embryo. The embryo is not harmed in the process and the cell can then be grown in the lab, circumventing ethical objections to hESC research which requires the destruction of embryos.

November 7, 2006: Missouri voters pass Amendment 2, a constitutional amendment that states that any hESC research or treatment allowed by the federal government will also be allowed in Missouri. The narrow victory (51%–49%) galvanizes opposition to the bill, much of which is centered on their contention that it would allow human cloning.

November 28, 2006: In the wake of the Hwang Woo-Suk scandal, a panel lead by John I. Brauman recommends changes in the procedures used to review papers submitted for publication in *Science*. The changes recommended include flagging high-visibility papers for further review, requiring authors to specify their individual contributions to a paper, and online publication of more of the raw data on which papers are based.

January 7, 2007: Dr. Anthony Atala of Wake Forest University and colleagues from Wake Forest and Harvard Universities report the discovery of amniotic– fluid-derived stem cells (AFS), which seem to hold similar promise to hESCs. The researchers reported that AFS could be extracted without harm to mother or child, thus avoiding some of the moral controversies regarding hESCs.

February 28, 2007: Governor Chet Culver of Iowa signs the "Iowa Stem Cell Research and Cures Initiative," a bill which ensures that Iowa researchers will be allowed to conduct stem cell research and that Iowa patients will have access to stem cures and therapies. The bill also prohibits human cloning.

March 31, 2007: New York passes a budget for the fiscal year 2008 that includes an appropriation of $100 million for stem cell and regenerative medicine research. The funds will be distributed through the Empire State Stem Cell Trust, which will be funded at $50 million per year for 10 years after the initial appropriation of $100 million.

April 11, 2007: Richard K. Burt and colleagues report success in treating type 1 diabetics in Brazil with stem cells taken from their own blood. The experimental procedure, reported in the *Journal of the American Medical Association*, has allowed the diabetics to stop taking insulin for as long as three years.

May 30, 2007: California Governor Arnold Schwarzenegger and Canada's Premier of Ontario Dalton McGuinty announce an agreement between Canada's International Regulome Consortium and the Stem Cell Center at the University of California, Berkeley, to coordinate research. McGuinty also announced the creation of the Cancer Stem Cell Consortium, which will coordinate and fund cancer stem cell research, and announced an initial donation of $30 million Canadian to the consortium from the Ontario Institute of Cancer Research.

June 6, 2007: Rudolf Jaenisch and colleagues at the Whitehead Institute, affiliated with the Massachusetts Institute of Technology in Boston, announce in *Nature* that they have succeeded in manipulating mature mouse stem cells so they have the properties of ESCs. In the same issue of *Nature*, Shinya Yamanaka and colleagues at Kyoto University announce that they have developed a method to reprogram stem cells in mice back to the embryonic state, so they may then develop into different body cells similarly to hESCs. If this technique is adaptable to human cells, it would allow researchers to bypass most of the controversy involved with the use of hESCs derived from human embryos.

June 20, 2007: President Bush vetoes legislation that would have allowed federal funding for ESC research using cells from embryos from fertility clinics that would be destroyed anyway. At the same time, Bush issues an executive order encouraging federal financial support of research aimed at creating stem cells without destroying embryos. The veto places him in opposition to most American voters and many members of the Republican Party. In response to the Bush veto, Democratic presidential candidates Hillary Clinton and Barack Obama pledge to support federal funding for hESC studies if elected.

August 3, 2007: Kitai Kim, George G. Daley and colleagues and Children's Hospital, Boston, report in the journal *Cell Stem Cell* that Hwang Woo-Suk, the discredited Korean researcher, did have one significant research result which appears to be genuine. The Children's researchers determined that Hwang's purposed ESCs were produced by parthenogenesis (virgin birth) from unfertilized eggs, a result since achieved by other researchers as well.
November 6, 2007: New Jersey voters reject a ballot measure which would have allowed the state to borrow $450 million to fund for stem cell research. Defeat of the initiative is attributed to the state's worsening fiscal condition and a vocal alliance of conservatives, antiabortion activists, and representatives of the Catholic Church who oppose stem cell research.

November 14, 2007: Shoukhrat Mitalipov and colleagues at the Oregon Health and Science University's national Primate Research Center announce in *Nature* that they have successfully derived ESCs by reprogramming genetic material from the skin cells of rhesus macaque monkeys.

November 20, 2007: The journals *Cell* and *Science* report on discoveries by two independent teams of scientists that reprogram human skin cells to have the characteristics of hESCs. One team is led by Shinya Yamanaka; the other is led by James Thomson of the University of Wisconsin, Madison.

2008: Rudolf Jaenisch and colleagues correct sickle cell anemia in mice using iPS cells.

January 14, 2008: Doris Taylor and colleagues at the University of Minnesota report success in creating a beating rat heart by injecting cells from newborn rats into the values and outer structure from a dead rat heart.

February 20, 2008: Scientists at Novocell, a private biotechnology company located in San Diego, announce that they have successfully used hESCs to control diabetes in mice whose own insulin producing cells had been destroyed.