THE THERAPEUTIC POTENTIAL OF STEM CELLS

FOR PARTIAL FULFILLMENT OF B.Sc. BIOTECHNOLOGY

REVIEW

BY

RICHA G. DESHPANDE

T.Y. B.Sc Biotechnology

UNDER THE GUIDANCE OF

Dr. ALPANA S. MOGHE

BHARATI VIDYAPEETH DEEMED UNIVERSITY

RAJIV GANDHI INSTITUTE OF IT AND BIOTECHNOLOGY,

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BHARATI VIDYAPEETH UNIVERSITY
RAJIV GANDHI INSTITUTE OF IT & BIOTECHNOLOGY, Katraj, Pune-46

CERTIFICATE

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This is to certify that **Ms. Richa G. Deshpande** of **Third year, B.Sc Biotechnology** has satisfactorily completed the REVIEW REPORT for fulfilment of Bachelor Degree is Biotechnology as prescribed in the syllabus of **Rajiv Gandhi Institute of IT and Biotechnology, B.V.D.U, Pune, in the academic year 2015-2016.**

Candidate Mrs. Alpana S. MogheDr. G.D. Sharma

Review Guide Principal

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1.0 INTRODUCTION

The human body comprises of over 200 different cell types that are organized into tissues and organs to provide all the functions required for viability and reproduction. In the mid-1800s itwas discovered that cells were basically the building blocks of life and that some cells had the ability to produce other cells. It all began in 1956 when bone marrow transplant was first successful to treat a patient with leukaemia, performed by Dr.E. Donnall Thomas in New York.

Stem cells, in general, are a population of undifferentiated renewable cells that can differentiate into tissue-specific cell types with specialized functions. Currently trending in therapeutics, stem cells are the best 'tools' to treat intractable degenerative or genetic diseases, due to their special capacity to engraft, differentiate, and generate new healthy cells to replace damaged or diseased cells in the body. Such stem cell candidates include Embryonic Stem Cells (ESCs), types of Adult Stem Cells (ASCs), and Induced-pluripotent Stem Cells (iPSCs). iPSCs are a unique class of stem cells, where cells from a wide range of tissues are "reprogrammed" using transcriptional factors, to become pluripotent. Another type of stem cells, called Cancer Stem Cells (CSCs), found to function in tumours, acting as a 'driving force' behind their development, right from the initial stage to metastasis.

Cell-based Therapies are treatments in which stem cells are used, based on their capacity to differentiate, to repair damaged or destroyed cells or tissues. Tissue Engineering (TE) is thepractice of combining scaffolds, cells, and biologically active molecules into functional tissues. It is an application of stem cells that follows the principles of cell transplantation, materials science, and engineering towards the development of biological substitutes that can restore and maintain normal function. Regenerative Medicine (RegMed) is a field of medicine that uses the techniques of TE and the phenomena of "self-healing", where the body the body uses its own systems, sometimes with thehelp of foreign biological material to recreate cells and rebuild tissues and organs. The terms "tissue engineering" and "regenerative medicine" have become largely interchangeable, as the field hopes to focus on cures instead of treatments for complex and chronic diseases.

An example of such cell-based therapies is the islet cell transplantation, done to treat patients suffering from Diabetes Mellitus, characterized by a deficit in ß-cell mass causing insufficient or impaired secretion of insulin. Pancreatic transplantation has been an effective treatment for Type-1 Diabetes, but due to limited organ and organ donor availability, surgical complications, and life-long immunosuppression, stem cells such as ESCs and ASCs are used as ideal sources of cells for Islet transplantation.

Stem cell research is exciting due to its tremendous potential to benefit human health and the opportunities of interdisciplinary research that it presents. This review gives an insight into the world of stem cells; the basics, properties, types and applications of stem cells in various stem-cell-based therapies. Emphasis is given on the potential role of the different stem cell types in Tissue Engineering (TE), Regenerative Medicine (RegMed) and treatment for Diabetes Mellitus.

2.0 STEM CELLS

A group of cells in our body with special capabilities of self-renewal and differentiation into various cell types are collectively termed as stem cells. Due to cellular heterogeneity within tissues such as blood, skin and intestinal epithelium, the differentiated cells have a short lifespan and are unable to self-renew. This led to the concept that such tissues are maintained by stem cells. The additional putative properties of stem cells include capacity for asymmetrical and infrequent division and any cell not exhibiting theseproperties is not considered as a stem cell.

There are 4 main types of stem cells obtained from different sources. They are: Embryonic stem cells, Adult/Mesenchymal stem cells, Cancer stem cells and Induced pluripotent stem cells.

2.1 PROPERTIES AND POTENCY OF STEM CELLS:

Although stem cells are present in small portions in the body, they possess special characteristics that are not exhibited by any other cell types.

Though they can be harvested from various sources, each of the four stem cell types has common characteristics mentioned below:

1) *Proliferation and Self-renewal:*Stem cells repeatedly proliferate by mitosis, implying that they can produce multiple copies of themselves over time. 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. After division, one of the daughter cells retains its stem cell properties, while the other daughter cell gets differentiated*(Figure-1)*.Embryonic stem cells appear to be able to proliferate for a year or even longer without ever developing into specialised cells, contrasting with adult cells.

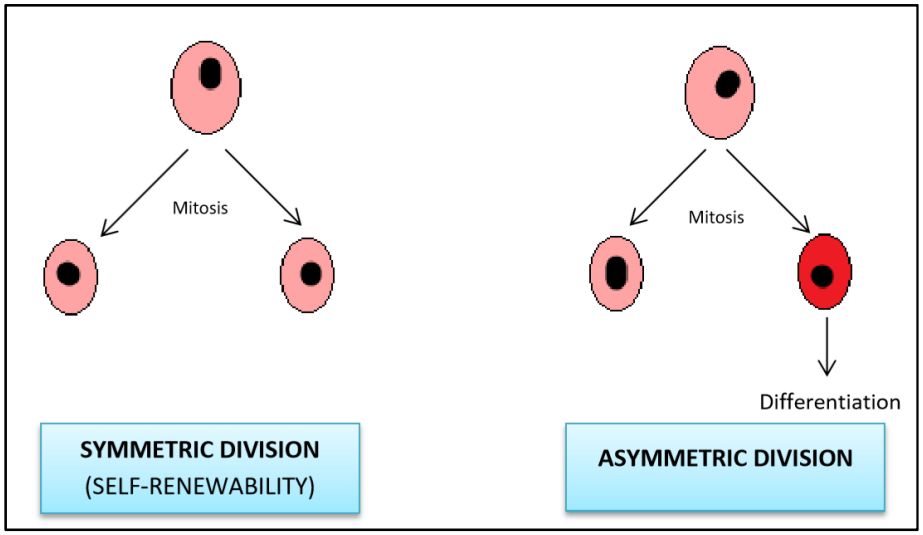

Figure-1 Proliferation of Stem Cells: Stem cells proliferate by mitosis, where, either both daughter cells are identical to the parent cell and retain the 'stemness' (Symmetric Division), or one daughter cell retains the stem cell properties of the parent cell and the other daughter cell becomes functionally differentiable (Asymmetric Division).

2) *Unspecialized Nature:*The unspecialised nature of stem cells means that stem cells lack the tissue-specific structures that allow them to perform specialised functions in the body. The heart muscle functions to pump blood through the body and nerve cells send signals to other nerve cells and the rest of the body, allowing movement. A stem call does not have a specialised function but it has the capacity to differentiate into a specialised cell that can carry out such functions.

3) *Differentiation:*When unspecialized stem cells give rise to specialized cells, the process is called differentiation. While differentiating, the cell usually goes through several stages, becoming more specialized at each step. The internal signals are controlled by a cell's genes, which are interspersed across long strands of DNA and carry coded instructions for all cellular structures and functions. The external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighbouring cells, and certain molecules in the microenvironment. The interaction of signals during differentiation causes the cell's DNA to acquire epigenetic marks that restrict DNA expression in the cell and can be passed on through cell division[23].

Potency is a term used to denote its ability to give rise to other cells in the body. Stem cells can be separated into several potency categories:

1) *Totipotency:* The ability of stem cells to develop into any type of cell present in the human body, or even give rise to a complete functional organism. For example, zygote, morula.

2) *Pluripotency:* The ability of stem cells to differentiate into any cell type originating from the three germ layers of the embryo, but cannot develop into a complete organism. They descend from totipotent stem cells.For example, embryonic stem cells.

3) *Multipotency:* The ability of stem cells to differentiate into many cell types within a specific type of tissue. They descend from pluripotent stem cells. For example, progenitor cells such as hematopoietic stem cells.

4) *Unipotency:* The ability of stem cells to give rise to a single cell type. They descend from multipotent stem cells.

2.2 TYPES OF STEM CELLS:

There are many types of stem cells, differing in their degree of differentiation and ability to self-renew.

2.2.1 EMBRYONIC STEM CELLS (ESCs):

The first entity of life, the fertilized egg, is totipotent, i.e. it has the ability to generate an entire organism. This capacity is retained by early progeny of the zygote up to the eight-cell stage of the morula. ESCs, as the name suggests, are derived from embryos. Most ESCs are derived from embryos that develop from eggs that have been fertilized in vitro, in an in vitro fertilization clinic, and then donated for research purposes with informed consent of doctors. They are not derived from eggs fertilized in a woman's body[23]. ESCs

are obtained from the inner cell mass of the blastocyst stage (when the embryo consists of 50-150 cells, 4-5 days after fertilization) of the human embryo[2] *(Figure-2)*. Cells of the inner cell mass are no longer totipotent, but retain the ability to differentiate into all cell types of the three dermal layers (ectoderm, endoderm and mesoderm).

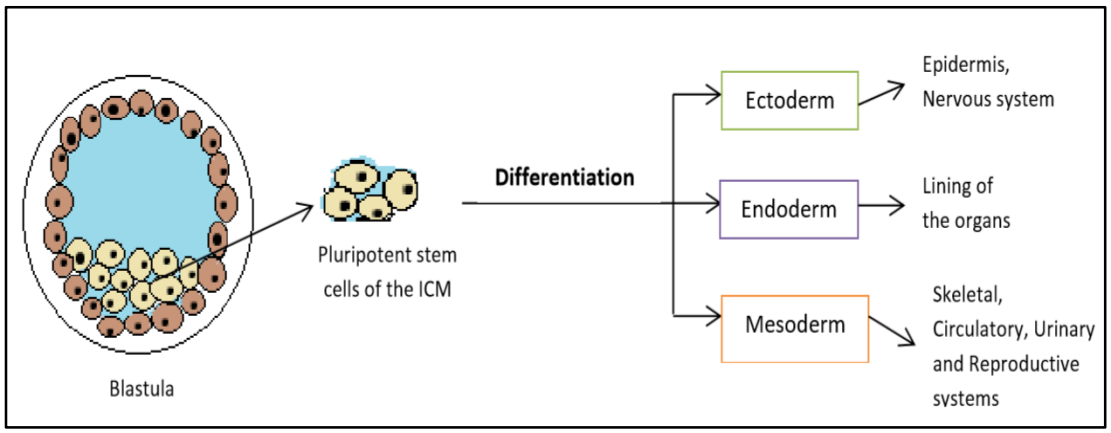

Figure-2. Pluripotency of Embryonic Stem Cells (ESCs): Pluripotent ESCs are obtained from the inner cell mass (ICM) of the blastula developed from an *in vitro*-fertilized zygote. Being pluripotent, these ESCs can differentiate into any cell type of the three germ layers, *i.e.*ectoderm, endoderm and mesoderm.

2.2.2 ADULT STEM CELLS (ASCs):

ASCs are the undifferentiated cells found among differentiated cells in a tissue or organ that can renew themselves and can differentiate to yield some or all of the major specialized cell types of the tissue or organ[23]. Depending on the source, they can either be multipotent or unipotent[3].

Prototypical ASCs, or mesenchymal stem cells (MSCs), have the capacity to differentiate into mesoderm and non-mesoderm derived tissues. Initially described in the bone marrow, MSCs are also found in most connective tissues of the body and can generate bone, cartilage, adipose tissues, cells supporting blood formation and fibrous connective tissue. The endogenous role for MSCs is maintenance of stem cell niches (classically, the hematopoietic). They also participate in organ homeostasis, wound healing and successful aging[3]. Sources of ASCs are illustrated in *Figure-3*.

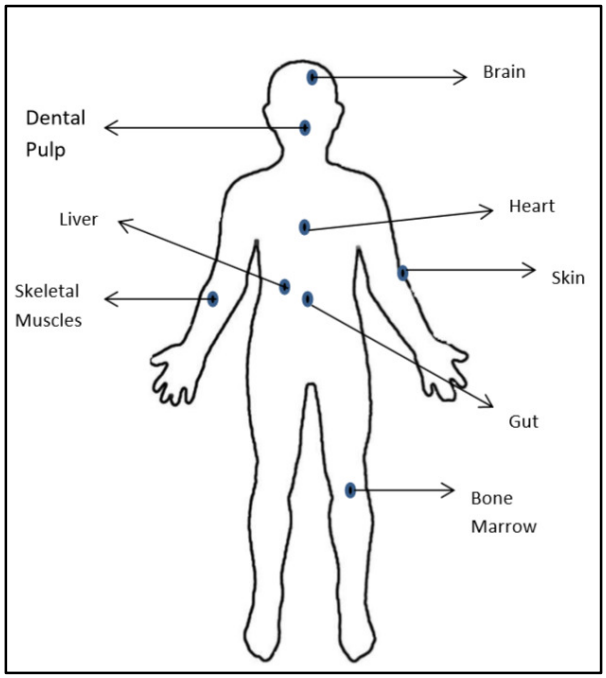

Figure-3. Sources of Adult Stem Cells (ASCs):ASCs are obtained from various parts of the human body, namely bone marrow, skin, adipose tissue, dental pulp, etc. On the basis of the source from the ASCs is derived, it is classified into different types such as hematopoietic stem cells (from the bone marrow), hepatic stem cells (from the liver), dental pulp stem cells (from the dental pulp), etc.

2.2.3 CANCER STEM CELLS (CSCs):

CSCs are the minority subpopulation of tumour cells endowed with properties such as self-renewal, differentiation and multipotency. They are also known as tumour initiating cells (TICs), or tumour propagating cells (TPCs), as they are capable of initiating tumour growth in case of certain cancer types[8]. They are specifically able to give rise to all cell types found in a cancer sample. Although less than 1% of the overall cancer cells have the ability to proliferate extensively and form tumours, they are the major reasons for the relapse of tumours, resistance to therapies, and metastasis[6]. CSCs are different from normal cells in the sense that they grow out of control. They, or their descendants, lost the behaviour of "contact inhibition of growth", which is the most important characteristic of normal cells.

Tumours are composed of heterogeneous cell subpopulations, defined by two different theories: the stochastic (or clonal evolution) model and the hierarchical model (or CSC hypothesis)[7]*(Figure-4)*. In the stochastic model, proposed by Peter Nowell in 1976, all tumour cells are biologically equivalent, with a similar capacity for self-renewal and formation of new tumour cells. Cell heterogeneity arises from sub-clonal differences resulting from genetic and/or epigenetic changes during cancer development. In the hierarchical model, only the TICs/TPCs are able to initiate tumour growth[6]. CSC-like properties may also be a function of various factors such as cell type origin, signals from the stromal microenvironment, accumulated somatic mutations,

and stage of malignant progression. The first evidence of CSCs came from the studies of human leukaemia in which researchers found that only a subset of leukemic cells can cause leukaemia when transplanted into a healthy body. Since then, cells with CSC characteristics have been discovered in a variety of humanmouse cancers, including breast, brain, and skin, prostrate and colonic cancers. In colon cancer, the CSCs present in the tumours are rare, whereas, in melanoma, a very large number of the tumour cells have CSC characteristics.

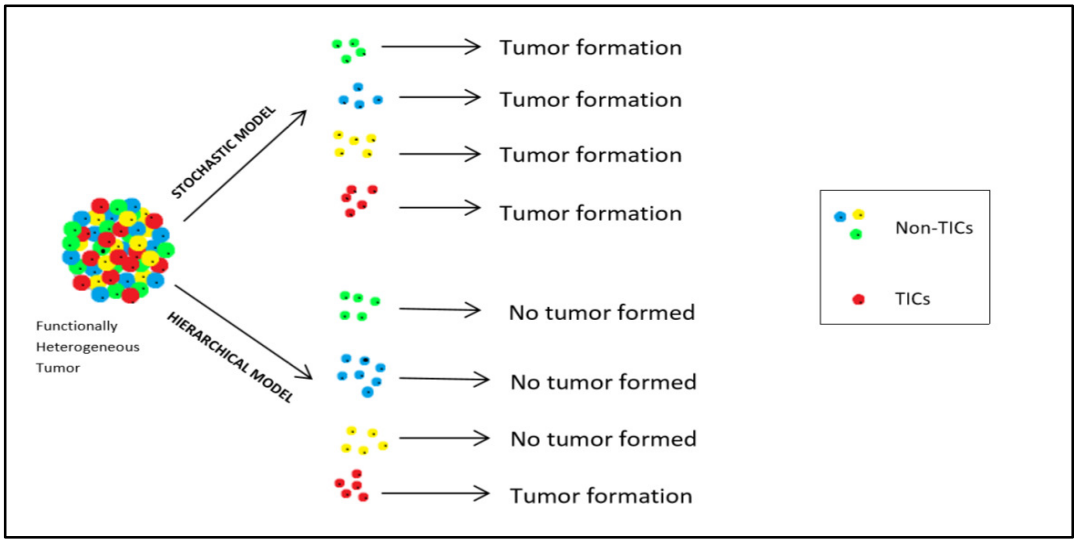

Figure-4. The Two Theories Defined for Cancer Stem Cells (CSCs): Tumours comprise of a heterogeneous population of Tumour-inducing cells (TICs) and Non-TICs. In the Stochastic Model, all the cells of the tumour are biologically equivalent and can proliferate into new tumour cells. In the Hierarchical Model, only the TICs are capable of initiating tumour growth.

2.2.4 INDUCED PLEURIPOTENT STEM CELLS (iPSCs):

Any cell of the body can be turned into a pluripotent stem cell by 'reprogramming'. Non-pluripotent stem cells can become iPSCs by inducing a "forced" expression of specific genes. These cells have properties similar to ESCs, such as the expression of genes and proteins for 'stemness', chromatin methylation patterns, doubling time, embryoid body formation, teratoma formation, viable chimera formation, and potency and differentiability. Cited as an important advance in stem cell research, iPSCs were first introduced by Shinya Yamanaka in 2006 from mouse cells. He isolated skin cells from a mouse model and injected four genetic factors (Oct4, Klf4, Sox2 and c-Myc). These factors attached to the genes which code for ESC proteins and overwhelmed the skin genes, 'fooling' the cells into thinking they are in an embryonic environment. As these cells replicate, they become increasingly similar to ESCs until they are completely reprogrammed to ESCs*(Figure-5)*. iPSCs have importance in research and therapeutics due to the controversial use of embryos for ESCs. Since they are obtained directly from the patient, they also avoid the graft-versus-host disease and immune rejection[24].

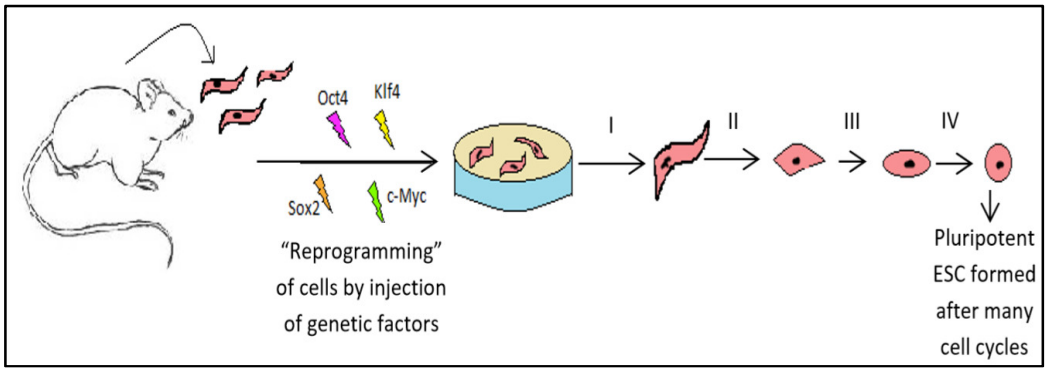

Figure-5. Development of Induced-pluripotent Stem Cells (iPSCs) by 'Reprogramming' Cells: Non-pluripotent stem cells can become iPSCs by a "forced" expression of specific genes by certain specific factors such as Oct4, Klf4, Sox2 and c-Myc. As these cells replicate, they become increasingly similar to ESCs, until they are completely programmed to ESCs.

2.3 APPLICATIONS OF STEM CELLS:

Stem cell research is a particularly interesting field. It has tremendous potential to benefit human health. Majority applications are in the field of research, to enhance the therapeutic potential of stem cells for human diseases. Some applications of stem cells are mentioned here.

2.3.1 STEM CELL THERAPIES:

Hematopoietic stem cell transplantation is the oldest and most widely available form of stem cell therapy. The source of stem cells is basically the bone marrow, while other sources include the peripheral blood or cord blood. The stem cells used can be allogenic or autologous. Allogenic stem cell transplantation is common for treating bone marrow failure and haematological malignancies (such as leukaemia)[1].

Stem cells have been used to generate epidermal tissue to provide autologous grafts to treat full-thickness wounds and severe burns[1].

Stem cell transplantation is also interlinked with gene therapy, bioengineering and immunology. Children with X-linked severe combined immunodeficiency were successfully treated with this combination of stem cell treatment and gene therapy. Another example is that of a patient suffering from epidermal blistering disorder, which was treated by an autologous graft of cultured epidermis in which the defective gene had been corrected *ex vivo*[1].

Other stem cell therapies involve tissue engineering and regenerative medicine, discussed later in *sections 3.0* and *4.0* respectively.

2.3.2 STEM CELL-DERIVED EXTRACELLULAR VESICLES:

Extracellular vesicles (EVs) from stem cells can recapitulate the therapeutic effects of stem cell transplantation. The hypothesis is that these EVs are effective by transferring biologically active molecules such as proteins, lipids, peptides, mRNA and microRNA from the stem cells to the injured or diseased cells. There have been studies to support the effect of ASCs through secretions, rather than differentiation mechanisms[9].

The hypothesis that stem cells could exert therapeutic activity through their secretions is highly possible as stem cell secretions include many biologically potent molecules such as growth factors, cytokines, chemokines, and bioactive lipids that could elicit wide-ranging physiological effects. MSC-conditioned culture medium alone has been reported to recapitulate the efficacy of MSCs in cardio-protection, renal tubular cell survival, protection against fulminant hepatic failure, and immunomodulatory activity to alleviate immune. Stem cells exert therapeutic activity through their secretions by communicating therapeutic signals from stem cells to recipient cells to initiate repair and regeneration. The first therapeutically efficacious EVs secreted by stem cells were reported in 2009 when Bruno *et al.* reported that 180 nm MSC-derived microvesicles protect against acute tubular injury. It was also reported that the smaller MSC-derived exosomes with a hydrodynamic radius of 55–65 nm also protect against acute myocardial ischemia/reperfusion injury, enhance wound healing, alleviate graft-versus-host diseases, reduce renal injury, and promote damaged hepatic regeneration. Xinetal. reported thatMSC-derivedexosomespromote neural plasticity and functional recovery in stroke via the transfer of miR-133b. Katsuda*et al.* reportedthathuman adiposeMSC-derivedexosomescontainfunctional neprilysin,amajor β-amyloid peptide-degrading enzyme and, thus, have the potential to reduce the pathological accumulation of β-amyloid peptide in Alzheimer's disease. MSC-derived EVs were also found to protect against hypoxia- and endotoxin-induced lung injury, and cardiovascular disease. Neuronal stem cells were first reported to secrete EVs in 2005 and were shown in 2013 to promote neural plasticity and functional recovery after treatment of stroke. Camussi*et al.* proposedthattheseEVscould be home to target cells through receptors present on their surface such that the cargoofEVsisloadedintodiseasedor injuredtargetcellstoinitiatetissuerepairandregeneration upon internalization[9].

2.3.3 CORD BLOOD BANKING:

After a baby is born, the cord blood is left in the umbilical cord and placenta, which are discarded as waste materials. It is easy to collect, with no risk to the mother or baby. Cord blood is known to be a useful source of hematopoietic stem cells (HSCs). Generally, HSCs are found exclusively in the bone marrow. HSCs can differentiate into any type of blood cell (RBCs, WBCs and platelets) and are also responsible for maintaining blood production throughout the life. Thus, they are widely used in treating blood-related diseases[24]*(Figure-6).*

Today, cord blood transplantation is offered to patients with cancerous blood disorders (such as leukaemia) or genetic blood diseases (like Fanconi anaemia). The transplanted HSCs make new, healthy blood cells to replace those damaged by the patient's disease or by a medical treatment. Cord blood transplant also offers a useful alternative to bone marrow transplants for some patients, as it is easier to collect than bone marrow and can be stored by cryopreservation methods until it is needed. It also is also less likely than bone marrow to cause immune rejection or complications, such as graft-versus-host disease. This implies that the cord blood need not be as perfectly matched to the patient as bone marrow, although some level of matching is still necessary.

A major drawback of cord blood transplantation is that the blood obtained from a single umbilical cord does not contain as many HSCs as compared to a bone marrow. Thus, cord blood has been used to treat mostly children, since the need for the HSCs would be less in comparison to an adult. A transplant containing too few HSCs may fail or could lead to slow formation of new blood in the body in the early days after transplantation. This has been partially overcome by transplanting blood from two umbilical cords into larger children and adults. Additional blood from the placenta is also collected in order to increase the total number of HSCs. Neither solution has been proven to be entirely satisfactory.

According to EuroStemCell, There are over 130 public cord blood banks in 35 countries, which are regulated by Governments and adhere to internationally agreed safety, quality and ethical standards[24].

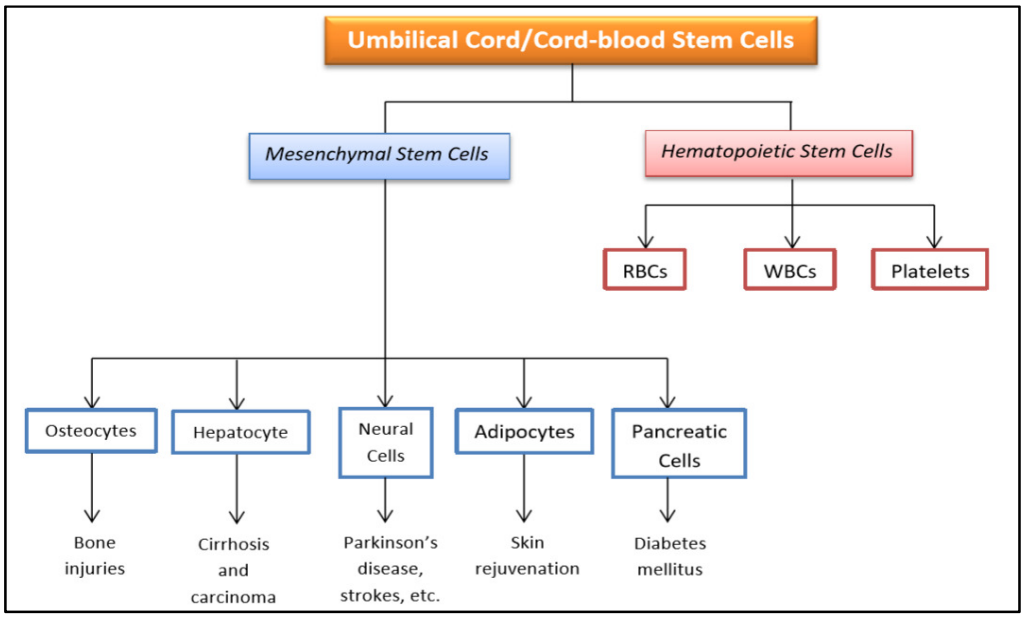

Figure-6. The Potential of Cord-blood Cells: Blood obtained from the umbilical cord or the placenta (often discarded as waste material) is an excellent source of Mesenchymal Stem Cells (MSCs) and Hematopoietic Stem Cells (HSCs), which are capable of differentiating into

a wide array of cell types. These cells can therefore be used for the treatment of blood-related and non-blood-related diseases.

2.3.4 SKIN GRAFTS:

The skin is the only tissue that is renewed throughout the life and this process involves a host of different stem cells. Skin stem cells are responsible for constant renewal of the skin, and for healing wounds. There are several types of skin stem cells such as:

1) *Epidermal Stem Cells:* Everyday regeneration of the different layers of the epidermis.
2) *Hair follicle Stem Cells:*Ensure constant renewal of the hair follicles, and can also regenerate the epidermis and sebaceous glands.
3) *Melanocyte Stem Cells:* Responsible for regeneration of melanocytes (that secrete melanin).

In 1970, Professor Howard Green in the USA, extracted epidermal stem cells from a patient, multiplied it and used it to grow sheets of epidermis in the lab. The new epidermis can then be transplanted back onto the patient as a skin graft. This technique is mainly used to save the lives of patients who have third degree burns over very large areas of their bodies[24].

2.3.5 DISEASE MODELLING:

Research is often limited by access to patients and availability of diseased tissues for study. A disease model is a representation of the abnormal human or animal biology that occurs in a particular disease. Instead of using animals as models, stem cells in a dish can serve as models. iPSCs offer a powerful tool for disease modelling. They act as a source of cells that are otherwise difficult to obtain, such as brain cells. An example of the use of iPSC disease models is in research on a currently incurable disease, called spinal muscular atrophy (SMA).

iPSCs have many advantages. They can be made by reprogramming the patient's own cells to make patient-specific cells in the laboratory and if the disease has a genetic cause, the lab-grown cells will carry that genetic flaw. Another advantage of using iPSCs is that they mature cells are reprogrammed to their embryonic selves. This enables scientists to model the cellular events in the diseased patients. This is also a valuable tool for searching for and testing new drugs[24]*(Figure.7).*

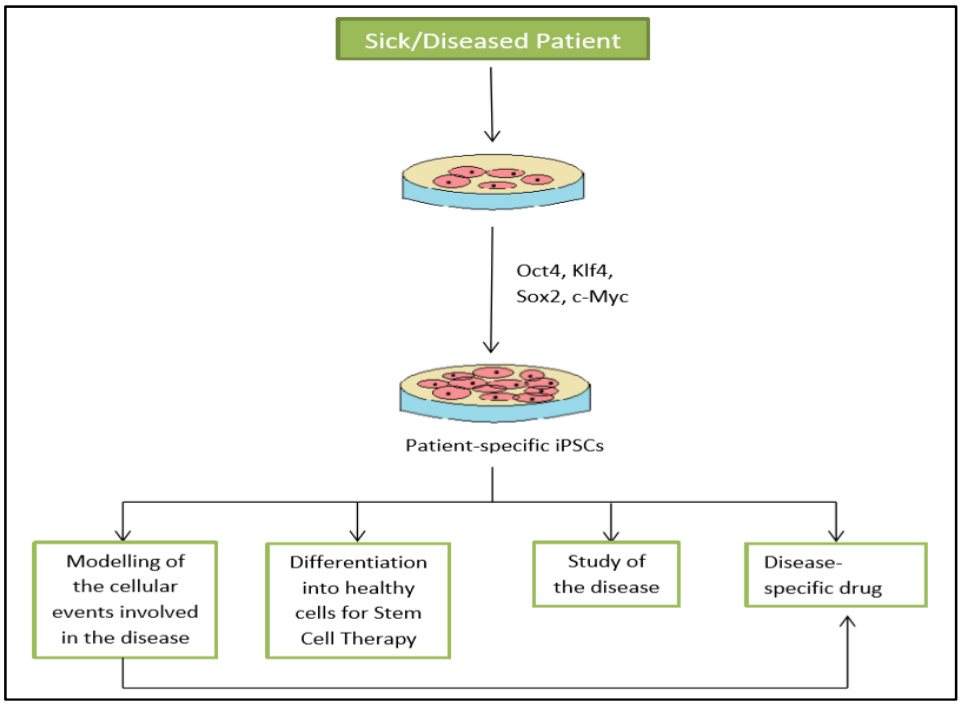

Figure-7. Advantages of Disease Models: Instead of using animals as disease models, stem-cell-based disease models serve as powerful tools. The patient's own cells can be used to make patient-specific disease models, which enable scientists and doctors to study the cellular events in the diseased patient, and accordingly design and test drugs specific for that disease.

2.3.6 POTENTIAL CURE FOR CURRENTLY UNCURABLE DISEASES:

1) *Alzheimer's Disease:* It is the most common cause of dementia. It is a complex neurodegenerative disease that affects nerve cells (neurons) in many parts of the brain. Transplantation of healthy, working neural stem cells into the diseased patient is currently under research. Another possible approach is to use stem cell types to deliver neurotrophin to the diseased brain to support the growth and survival of the neurons[24].

2) *Cerebral Palsy:* It is a collective term to describe the effects of damage to a developing brain. The damage can occur early on during brain development, either in the baby during pregnancy or during the period soon after birth. One idea was that neural precursor cells could be transplanted into patients or used to make new nerve cells in the lab to replace lost cells in the patient's brain. Another alternative was to inject mesenchymal stem cells or cord blood stem cells to help protect or repair damaged nerve cells[24].

3) *Huntington's Disease:* It is a hereditary neurodegenerative disease that mainly affects nerve cells in the brain called medium spiny neurons (MSNs). Scientists hope to be able to use stem cells (ESCs or iPSCs) to

grow new, healthy MSNs that can be transplanted into patients to replace cells that are damaged and destroyed[24].

4) *Kidney Diseases:* It usually involves damage to the nephrons and can be acute or chronic. A group of cells at the urinary pole of the Bowman's capsule of the nephron have been discovered to have key features of stem cells and are responsible for production of podocytes, which are specialised cells involved in the filtration work of the nephron and that need to be replaced continuously throughout the life.Mesenchymal stem cells may release proteins that can help kidney cells to grow, inhibit cell death and encourage the kidney's own stem cells to repair kidney damage.iPSC can be used to make the glomerulus and tubules, the building blocks of the nephron[24].

5) *Multiple Sclerosis:* It is a disease that affects nerve cells in the brain and spinal cord. It may be possible to use mesenchymal or blood stem cells to 'reset' the immune system to prevent it from attacking the nerve cells, or reduce the amount of damage done. Also, these stem cells may be able to help repair the damaged myelin sheath, 'remyelinating' the nerves and allow them to function correctly again, which could prevent the nerves themselves from degenerating[24].

6) *Muscular Dystrophy:* It is a group of genetic diseases that affect skeletal muscles and often also heart muscle. Current research is focussed stem cells (myoblasts and mesoangioblasts) to restore production of the missing protein dystrophin in the muscles of patients[24].

3.0 TISSUE ENGINEERING

3.1 WHAT IS TISSUE ENGINEERING?

Tissue and organ damage, degeneration and failure, caused due to diseases, injury or trauma necessitates treatments to facilitate their repair, replacement or regeneration. Treatment typically focuses on transplanting tissue from one site to another in the same patient (known as an autograft) or from one individual to another (known as a transplant or allograft), surgical repair, artificial prostheses, mechanical devices and sometime, drug therapy. Although these treatments have proven to be revolutionizing and life-saving, they are associated with major problems. Harvesting autografts is expensive, painful, constrained by anatomical limitations and associated with donor-site morbidity due to infection and hematoma. Similarly, allografts and transplants also have serious constraints due to problems with accessing enough tissue for all of the patients who require them and the fact that there are risks of graft-versus-host diseases, immune rejection and infections introduced from the donor to the patient[13].

In 1988, National Science Foundation defined tissue engineering (TE) as a technique that aims at regenerating damaged tissues, instead of replacing them. It is the application of principles and methods of engineering and life-sciences toward the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain or improve tissue functioning[13]. Artificial skin and cartilage are examples of engineered tissues that have been approved by the FDA; however, currently they have limited use in human patients[25]. The idea of replacing damaged tissue with another dates as far back as the 16th century, when Italian surgeon and professor GaspareTagliacozzi at the University of Bologna, described a nose replacement that he had constructed from a forearm flap in his work 'De Custorum Chirurigia per Insitionem' (The Surgery of Defects by Implantation), which was published in 1597. Today, the field of TE is a highly multidisciplinary one and draws in experts from clinical medicine, mechanical engineering, materials science, genetics, and related disciplines[13].

TE strategies generally fall into two categories:

1) *Acellular Matrices:* Only matrices are used and depend on the body's natural ability to regenerate for proper orientation and direction of new tissue growth. Such matrices are usually prepared by removing cellular components from tissues via mechanical and chemical manipulation to produce collagen-rich matrices. They tend to eventually degrade on implantation and are generally replaced by the extra-cellular matrix (ECM) proteins secreted by the ingrowing cells[12].

2) *Matrices with Cells:* Cells dissociated from a small piece of donor tissue are either implanted directly into the host or are expanded in culture, attached to a support matrix, and then replanted into the host after expansion. The sources of donor tissues can be heterologous (such as bovine), allogenic (another human donor), or autologous (from the host itself)[12].

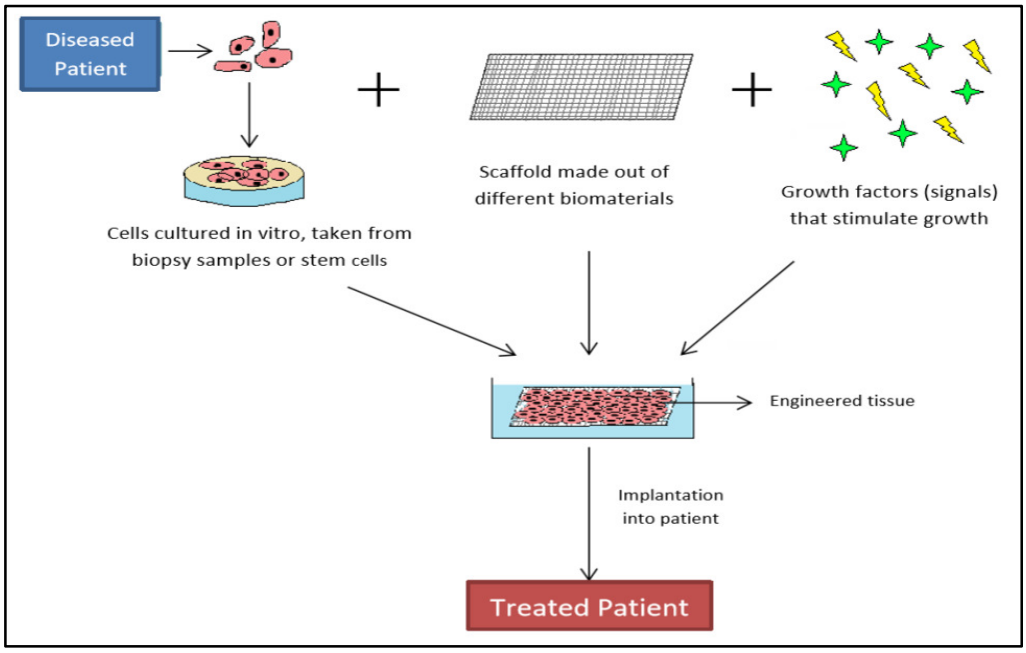

Figure-8. The Therapeutic Application of Tissue Engineering (TE): Tissues can be reconstructed *in vitro* with by using cell cultures (from a biopsy sample from the patient, or from healthy stem cells of the patient which are induced to become pluripotent, or healthy stem cells from a donor which are induced to become pluripotent), scaffolds made of special biomaterials, and certain necessary growth factors and signals that stimulate growth and provide the niche for the cells.

3.2 STEPS INVOLVED:

TE involves a combination of cells, signals and the scaffold, often referred to as a tissue engineering triad*(Figure-8)*.

1) *Cells:* Most current TE strategies depend upon a sample of autologous cells from the diseased organ of the host by performing tissue biopsies. In some cases where patients experience extensive end-stage organ failures, the biopsy may not yield enough normal cells for expansion and transplantation. Primary autologous human cells cannot be expanded from certain organs, like the pancreas. In such situations, stem cells are used and a viable source of cells. They serve as an alternate source of cells from which the tissue can be reconstructed. ESCs and iPSCs are used due to their property of pluripotency[12].

2) *Scaffolds:* A scaffold is a structure of artificial or natural biomaterials which act as templates for tissues to grow and provide a natural environment for tissue or organ regeneration. Since majority of mammalian cell types are anchorage-dependant and will die if no cell-adhesion substrate is available, these biomaterials forming the 3D scaffold provide a cell-adhesion substrate that can deliver cells to specific sites in the body with high loading efficiency[12]. They can be derived from donor tissue or made from natural/synthetic polymers known for their strength and endurance. Sometimes the scaffolds dissolve overtime and some are made to provide support to the regenerating organ. Typically, three individual groups of

biomaterials are used in the fabrication of scaffolds for TE- ceramics, synthetic polymers and biological materials. The scaffolds are seeded with cells *in vitro*.

3) *Signals:* The cell-seeded scaffolds are subjected to biophysical stimuli in the form of bioreactors, a device or system that mimics the *in vivo* environment by applying different types of mechanical or chemical (growth factors) stimuli to cells[13]. In some cases, bioactive signals and molecules, such as cell-adhesion peptides and growth factors, are loaded during cell-seeding to aid in cellular adhesion to the scaffold and regulate normal cell functioning.

4) The TE triad forms the engineered tissue, which is then implanted surgically into or topically onto the damaged site.

3.3 BIOMATERIALS:

According to the European Society for Biomaterials (ESB), a biomaterial is defined as the material intended to interface with biological systems to evaluate, treat, augment or replace any organ, tissue or function of the body. Biomaterials not only interact with the body, but also influence biological processes toward the goal of tissue regeneration[13].

Biomaterials are considered 'ideal' on the following basis:

1) It must be biodegradable and biocompatible to support the replacement of normal tissue without inflammation. Incompatibility causes inflammatory or immune response that eventually leads to rejection and/or necrosis.
2) It should provide and environment that allows appropriate cell behaviour regulation (adhesion, proliferation, migration, differentiation, etc.) occurs and the functional tissue can form.
3) It must serve as a temporary mechanical support which allows tissue growth in three dimensions, while the cells undergo spatial tissue reorganization.
4) During early development, it should allow the engineered tissue to maintain sufficient mechanical integrity to support itself.
5) During late development, it should have progressed in degradation such that it does not hinder further growth[13].

In accordance to the above mentioned criteria, three groups of biomaterials have been used in the fabrication scaffold for TE*(Table-1)*:

1) *Ceramics:* Such scaffolds are characterized by increased mechanical stiffness, very low elasticity and hard brittle surface, and hence, are not used for regeneration of soft tissues. It is mostly used for bone regeneration due to their chemical and structural similarity to the mineral phase of the native bone. Ceramics are also known for its capability to enhance osteoblast differentiation and proliferation, and thus, it has been used in dental and orthopaedic surgery to fill bone defects and improve implant integration with the host bone. Its disadvantages are its brittleness, difficulty of shaping for implantation, and moreover, difficulty in controlling degradation rates. Examples of ceramic scaffolds are hydroxy-apatite (HA) and tri-calcium phosphate (TCP)[13].

2) *Synthetic Polymers:* These biomaterials are polyesters of naturally occurring α-hydroxy acids, and have FDA approval for use in sutures.These polymers degrade by non-enzymatichydrolysis to give non-toxic, natural metabolites which are

eliminated from the body in the form of carbon dioxide and water. The degradation characteristics can be controlled by varying the polymer itself or the composition of the individual polymer. They are thermoplastic and so, can be fabricated with a tailored architecture to formed into a 3D scaffold. Drawbacks include reduced bioactivity causing risks of rejection, and cell and tissue necrosis due to the lowering of pH by the carbon dioxide formed during degradation. Examples of synthetic polymers commonly used are polystyrene, poly-L-lactic acid (PLLA), polyglycolic acid (PGA), and poly-D,L-lactic-co-glycolic acid (PLGA)[12][13].

3) *Biological Polymers:* These polymers are biologically active, and promote cell adhesion and growth. Being biodegradable, they allow host cells to produce their own extracellular matrix over time and replace the degraded scaffold. One such material is collagen, which contains cell-adhesion domain sequences that allow specific cellular interactions, which may assist to retain the phenotype and activity of fibroblasts and chondrocytes. Alginate (a polysaccharide isolated from seaweed) has gentle gelling properties in the presence of divalent ions such as calcium. It has been used as an injectable cell-delivery vehicle and a cell-immobilization matrix, and approved by the FDA as wound dressing material. However, the scaffolds formed from such biomaterials pose a challenge and have poor mechanical properties (which limits their use in many load-bearing orthopaedic applications)[12][13]. In addition, they can induce inflammatory responses because of undefined factors that cannot be eliminated by purification prior to implantation and pathogen transfer. Other problems are the significant degree of variability among different lots and the need for large-scale sources, particularlywhen human proteins are involved[22].

	Ceramics	Synthetic Polymers	Biological Polymers
Physical Properties	Increased stiffness	Thermoplastic and can therefore form 3D scaffolds; degrade to form non-toxic metabolites	Biodegradable
Drawbacks	Low elasticity and brittleness; not easily degradable	Reduced bioactivity; lowering of pH during degradation causing necrosis	For mechanical properties
Uses	Orthopaedic implants	Sutures	Cell-delivery vehicle, cell-immobilization matrix, wound-dressing
Examples	HA, TCP	PLLA,PGA and PLGA	Collagen, alginate

Table-1. Features of the Biomaterials Used in TE

3.4APPLICATIONS OF TISSUE ENGINEERING (TE):

1) Tissue grafts used for transplantation and preservation of transfusable blood products were developed by the company LifeCell. It also developed engineered porcine heart valves for replacement surgery in humans[11].

2) In the armed forces, battlefield-related skin injuries and chemical burns were treated using engineered human dermal tissue combined with a synthetic epidermal layer. The engineered tissue, known as Dermagraft-TC, covers and protects burns, helping to minimize infections and retain fluids until a sufficient amount of the patient's own skin is available for autografting[11].

3) TE products are also used in the toxicology testing and *in vitro* markets, as alternatives to certain types of animal testing. In 1995, Stratum Laboratories (La Jolla, CA) of the In Vitro Laboratory Testing (IVLT) business of Advanced Tissue Sciences, manufactured and sold living human skin tissue (known as Skin2) *in vitro* lab-testing kits used to test skin care, household, chemical and pharmaceutical products for a variety of indications[11].

4) Wound repair is the key application for TE products. Dermagraft has been applied to foot ulcers that develop as a side-effect of long-term diabetes[11].

5) Knee damage was repaired using tissue-engineered cartilage tissue made of autologous cultured chondrocytes.

6) TE has been successfully used in the regeneration of urethral tissue, reconstruction of bladder tissue, male and female genital tissue reconstruction, vascular graft development, and trachea/cartilage construction.

7) In addition to medical applications, non-therapeutic applications include using tissues as biosensors to detect biological or chemical threat agents, and tissue chips that can be used to test the toxicity of an experimental medication[25].

8) TE is the major component of regenerative medicine *(discussed in section 4.0).*

4.0 REGENERATIVE MEDICINE

4.1 WHAT IS REGENERATIVE MEDICINE (RegMed)?

The term 'regeneration' means the regrowth of a damaged or missing organ part from the remaining tissue*(Figure-9)*. As adults, humans can regenerate some organs, such as the liver and skin. If part of the liver is lost by disease or injury, the liver grows back to its original size, though not its original shape. And our skin is constantly being renewed and repaired[24]. RegMed is a broad field that includes tissue engineering but also incorporates self-healing, where the body uses its own systems, sometimes with help foreign biological material, to recreate cells and rebuild tissues and organs[25]. The goal of regenerative medicine is to find ways to initiate tissue regeneration in the body, or to engineer replacement tissues. The underlying principle involves taking interventional measures to stimulate innate healing mechanisms, which ultimately involve the immune system[14]. The terms "tissue engineering" and "regenerativemedicine" have become largely interchangeable, as the field hopes to focus on cures instead of treatments for complex, often chronic, diseases[25].

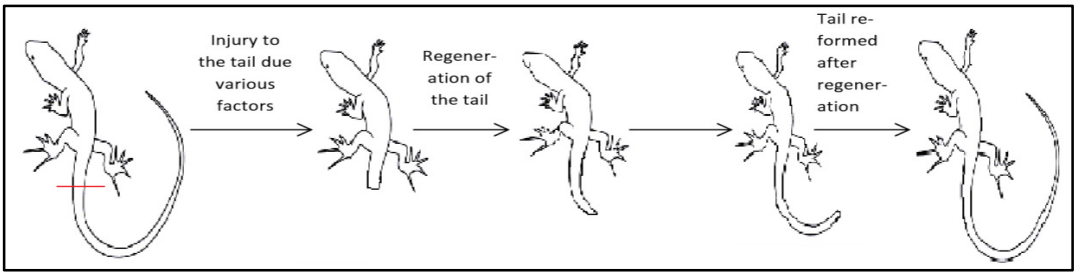

Figure-9. The Concept of Regeneration: The lizard has the capacity to self-heal when its tail is broken off due to injury. This gradual regeneration enables the lizard to regrow the tail.

4.2 RegMed AND STEM CELLS:

RegMed is a field of medicine devoted to cell-based therapies in which stem cells are used (or induced) to differentiate into specific cell types required to repair damaged or destroyed cell population or tissues. Stem cells are the primitive cells that have the capacity to differentiate into a variety of cell types when induced. They offer the potential to restore function to the damaged tissue by way of inducing the growth of new tissue that can be specialized for their respective functions. ESCs and iPSCs are the best candidates for RegMed, considering their capability of auto-reproducibility and pluripotency. Despite of their therapeutic potential, they have certain limitations associated, such as cellular regulation of teratoma formation, ethical consideration, source and immune concerns regarding ESCs, and difficulties with genetic manipulation with respect to iPSCs. In contrast, multipotent ASCs or MSCs are immunocompatible and also have no ethical concerns related to their use[15].

Certain criteria were proposed for stem cells in order to be considered as a viable resource for clinical regenerative therapies:

1) Ability to be collected in abundance.

2) Ability to be collected with a minimally invasive procedure.
3) Differentiability into a variety of tissue types in a regulatable and reproducible manner.
4) Ability to be transplanted to either an autologous or allogenic host in a safe and effective manner[15].

Considering the above criteria, MSCs derived from adipose tissue, known as adipose-derived stem cells (AdSCs), were seen to be an ideal population of cells *(Figure-10)*. AdSCs can be obtained in large numbers and easily harvested from adipose tissue. It is ubiquitously available and can differentiate into both, ectodermal and tissues and organs, although AdSCs originate from mesenchymal lineages[10]. They also exhibit low (or no) immunogenity after allogenic transplantation[15].

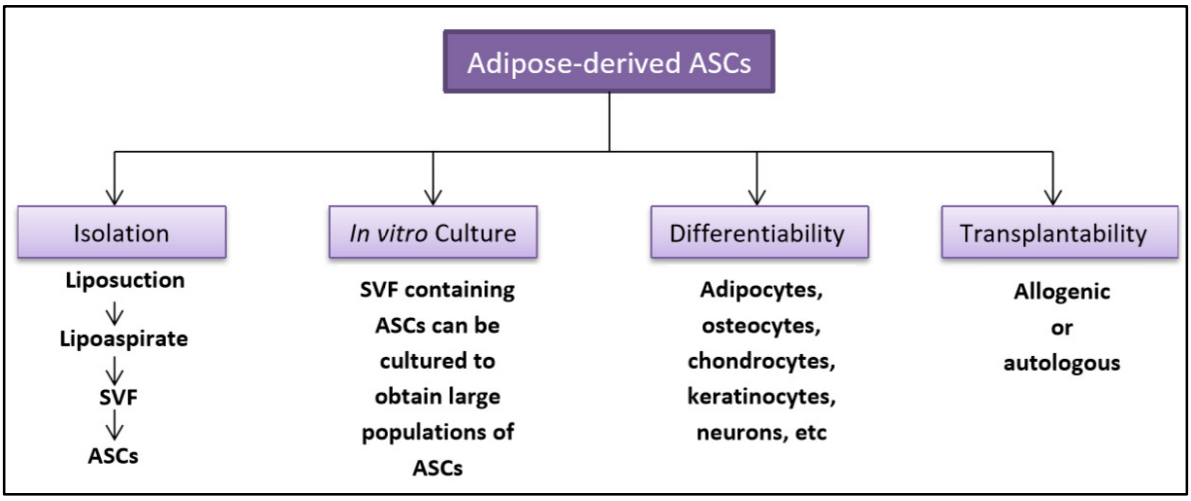

Figure-10.Adipose-derived ASCs (AdSCs) as an Ideal Cell-type for Regeneration: AdSCs are easily isolated by liposuction. It is present in the stromal vascular fraction (SVF) obtained after centrifugation of the lipoaspirate. The AdSCs proliferate quickly to produce large populations of AdSCs in vitro, which can later be used for regenerative medicine or for other therapeutic applications due to its multipotency.

4.3 THE REGENERATIVE NICHE:

The stem cells used for TE bear significant importance as the functional units of growth and regeneration in many tissues and cells, and maintaining proper tissue function. Hence, these cells must be protected from any forms of damage. At the same time, they must be able to provide effective responses to cues for cell replacement and/or repair. The stem cells must thus be maintained in a specialized microenvironment that provides the necessary spatial and temporal cues to support and coordinate stem cell activity[16]. The essential features of the regenerative niche are:

1) *It is tissue-specific*. For example, the niche in the liver is different from the niche in the central nervous system (CNS). Thus, the regenerative strategies designed for one niche may not necessarily apply to the other.

2) *It is highly complex*. Its regulation depends on activating and inhibitory factors that have autocrine (acting on the same cell), paracrine (acting on another cell) or endocrine (acting via the circulation on a distant tissue) effects.

3) *It is highly heterogeneous*. There are specific extra-cellular matrix components in addition to local progenitor cells and associated niche cells.

4) *It is highly dynamic*. As activating and inhibitory signal molecules are released, the oxygen levels fluctuate according to the state of integrity of the vascular systems, and the populations of cells keep increasing and decreasing. Control of these populations is exerted by processes such as proliferation, differentiation, apoptosis and necrosis. Necrosis generally occurs due to poor vascularization and/or infections[14].

Due to the above mentioned complexities of the niche, it is understood that it is a challenge to establish reproducible models, which adequately represent essential features of the required niche, and are not complex to such an extent that interpretation of the generated data is impossible. To solve the problem of in vitro modelling, heterotypic co-culture models have been developed. These models involve the culturing of more than one cell phenotype in a system which permits cellular cross-talks (communication between the different cell types). This method has been proven useful in vascularization, where adequate blood vessel networks are absolutely essential for providing oxygen and nutrients, and also to remove metabolic products from the tissue[14].

5.0 DIABETES MELLITUS

5.1 WHAT IT IS:

According to the World Health Organization (WHO) 2014 statistical report, 9% of adults, 18 years and older, had diabetes. In 2012 diabetes was the direct cause of 1.5 million deaths and more than 80% of those deaths occurred in low- and middle-income countries[25]. According to the 2012 Public Health Foundation of India (PHFI), India is presently home to 62 million diabetics, out of which 34 million are rural Indians while the remaining 28 million are urban Indians. Preliminary results from a large community study conducted by the Indian Council of Medical research (ICMR) showed that a lower proportion of the population is affected in states of Northern India (0.12 million in Chandigarh, 0.96 million in Jharkhand) as compared to Maharashtra (9.2 million) and Tamil Nadu (4.8 million). The National Urban Survey conducted across various cities of India reported estimates of% 11.7% in Kolkata (Eastern India), 6.1 % in Kashmir Valley (Northern India), 11.6% inNew Delhi(Northern India), and 9.3 % in West India (Mumbai) compared with South Indian states; 13.5 % in Chennai, 16.6 % in Hyderabad, and 12.4 % in Bengaluru[27] *(Figure-11)*.Thus, diabetes affects millions of people and is a very serious lifelong healthproblem[22].

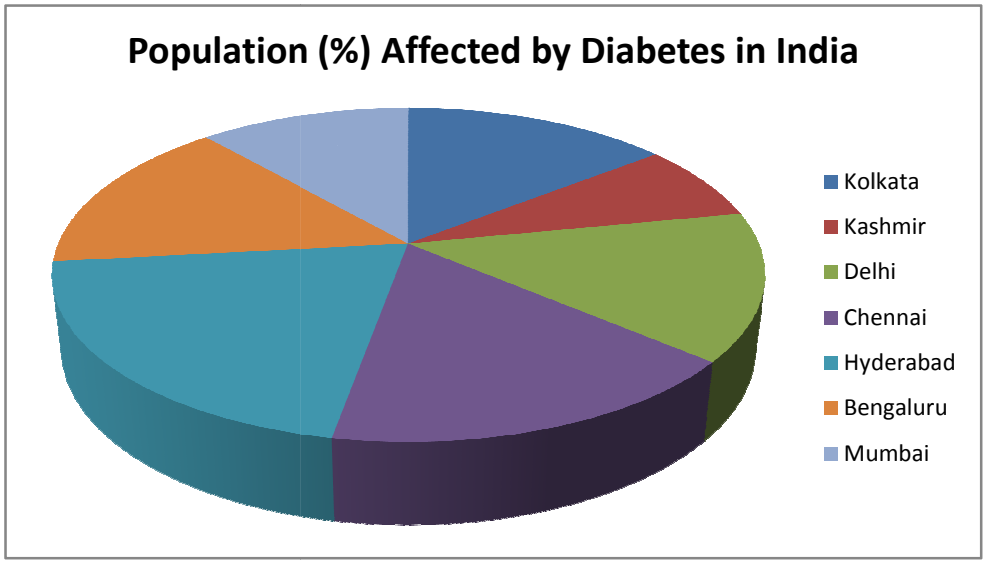

Figure-11.Population (%) Affected by Diabetes in India: 11.7% in Kolkata, 6.1 % in Kashmir, 11.6% in Delhi, 13.5% in Chennai, 16.6% in Hyderabad, 12.4% in Bengaluru, and 9.3% in Mumbai.

According to WHO, diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces.Insulin is one of the many hormones that regulate blood sugar. It is secreted by the ß-cells present in the pancreas. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the eyes, kidneys, heart, nerves and blood vessels[25].

5.2 COMPLICATIONS:

Hyperglycaemia has certain marked symptoms, including polyuria, polydipsia, weight loss and blurred vision. Chronic hyperglycaemia may also be accompanied by impairment of growth and susceptibility to certain infections[17].

Long-term complications of diabetes are:

1) Retinopathy with potential loss of vision
2) Neuropathy leading to renal failure
3) Peripheral neuropathy with foot ulcers, amputations, etc.
4) Autonomic neuropathy causing gastrointestinal, genitourinary, cardiovascular functions and sexual dysfunction
5) Atherosclerotic cardiovascular, peripheral arterial and cerebrovascular diseases
6) Hypertension
7) Lipoprotein metabolism abnormalities[17]

5.3CLASSIFICATION:

Assigning a type of diabetes to an individual depends on the circumstances present at the time of diagnosis. Many diabetic individuals do not fit into a single class and thus, it is important to understand the pathogenesis of the hyperglycaemia and treat it effectively. Majority of the cases fall into two broad categories, mainly, Type-1 and Type-2 diabetes[17]. There are other specific types, discussed below*(Figure-12)*.

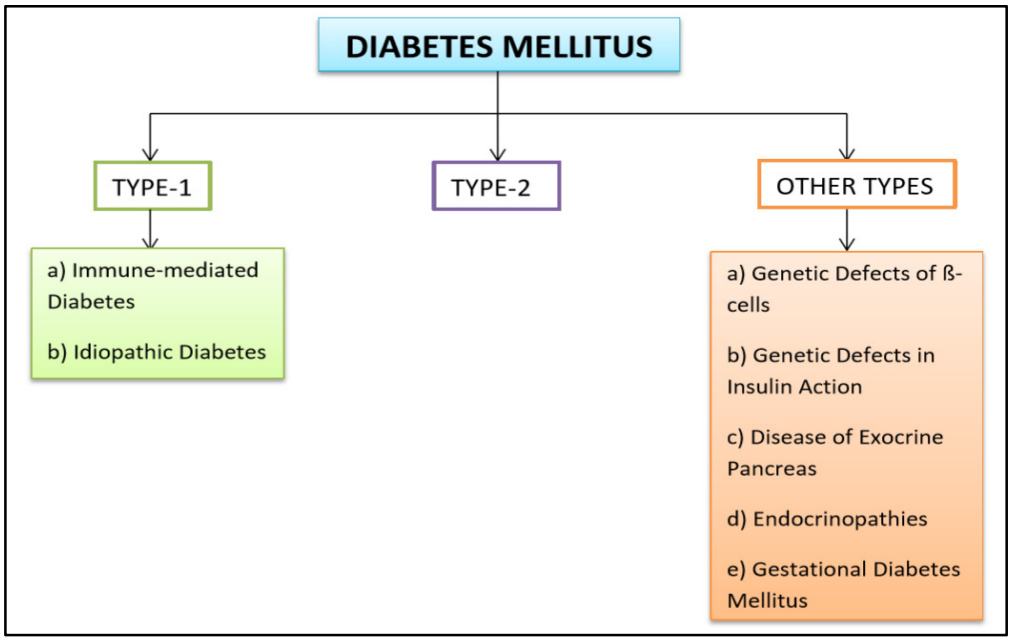

Figure-12. Classification of Diabetes Mellitus (DM): There are two main classes of DM, namely, Type-1 and Type-2 DM. Other specific types of DM include Gestational DM, Endocrinopathies, etc. They have lower occurrence rates as compared to Type-1 and Type-2 DM.

5.3.1 TYPE-1 DIABETES:

1) *Immune-mediated diabetes:* This type of diabetes is also described as insulin-dependent diabetes or juvenile-onset diabetes. It accounts for 5-10% of those with diabetes. It is caused due to cell-mediated autoimmune destruction of the pancreatic ß-cells. The rate of ß-cell destruction is quite variable, being fast in individuals (infants and children), and slow in others (adults). Some have modest fasting hyperglycaemia that can rapidly change to severe hyperglycaemia and/or ketoacidosis in the presence of infection or other stress. Adults may retain residual ß-cell function sufficient to prevent ketoacidosis, but, are at risk of ketoacidosis in later stages of life due to little or no insulin secretion. Patients with this type of Type-1 diabetes are at potential risk of autoimmune disorders such as pernicious anaemia, Hashimoto's thyroiditis and vitiligo[17].

2) *Idiopathic diabetes:* Patients with this form of Type-1 diabetes have permanent insulinopenia and are prone to ketoacidosis, but do not suffer from autoimmunity. Many African and Asian patients fall into this category of diabetes. It is strongly inherited, lacks immunological proof of ß-cell autoimmunity and demands absolute insulin-replacement therapy in affected patients[17].

5.3.2 TYPE-2 DIABETES:

This type of diabetes is also referred to as non-insulin-dependent or adult-onset diabetes and encompasses about 90-95% of those with diabetes. In such cases, individuals have insulin resistance and have relative insulin deficiency. Often throughout their lifetime, these individuals do not require insulin treatment to survive. Obesity is often observed in these individuals, which in itself causes some degree of insulin resistance. Ketoacidosis occurs spontaneously and it usually arises along with infections. Such patients are at increased risks of developing macrovascular and microvascular complications. Insulin secretion is defective in the patients and insufficient to compensate for insulin resistance. The risks of developing Type-2 diabetes increases with age, obesity and lack of physical activity[17].

5.3.3 OTHER TYPES:

1) *Genetic Defects of ß-cells:* Referred to as Maturity-onset Diabetes of the Young (or MODY), this diabetes is characterized by the onset of hyperglycaemia and impaired insulin secretion with minimal or no defects in insulin action. It is seen generally in young adults below 25 years of age and is inherited in an autosomal dominant pattern[17].

2) *Genetic Defects in Insulin Action:* Diabetes resulting from metabolic abnormalities associated with mutations of the insulin receptor. This syndrome was initially termed as Type-A insulin resistance. So far, two paediatric syndromes, Leprechaunism and Rabson-Mendenhall syndrome have been identified to have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance[17].

3) *Diseases of the Exocrine Pancreas:* Acquired processes such as pancreatitis, trauma, infection, pancreatectomy and pancreatic carcinoma can also cause diabetes[17].

4) *Endocrinopathies:* Hormones such as growth hormones, cortisol, glucagon and epinephrine antagonize insulin action. Excess amounts of these hormones and conditions such as somatostatinoma-induced and aldosteronoma-induced hypokalaemia can cause diabetes[17].

5) *Gestational Diabetes Mellitus (GDM):* Degree of glucose intolerance with onset or first recognition during pregnancy. Deterioration of glucose tolerance occurs normally during pregnancy, specifically in the 3rd trimester[17].

5.4 CURRENT RESEARCH AND STEM CELL THERAPY:

Considering the complications mentioned in *section 5.2*, emphasis is given on the importance of strict glycaemic control in order to reduce the ophthalmologic, neurological and renal complications associated with diabetes mellitus[20]. In many cases, diabetic drug therapies are unable to provide sufficient strict control of blood glucose. Pancreas transplantation from donors is an effective form of treatment, but, it has major disadvantages of major surgeries, lifetime immunosuppression, and high cost and unavailability healthy donors[19]. A curative therapy was the replacement of the pancreatic cells and islet cells. An unlimited source of such cell replacement therapy was reported to be the ß-cell lines derived from rodents. Another potential source for cell replacement therapy is stem cells. A more detailed explanation is mentioned below.

5.4.1 ISLET CELL TRANSPLANTATION:

Allogenic islet transplantations have been considered as an effective treatment for Type-1 diabetes. Islet cells from the donor pancreas are extracted and injected into the portal vein of the patient's liver. However, shortage of donor material and the need for immense immunosuppression to prevent graft rejection are major limitations. This treatment is much less successful than pancreatic transplantation at accomplishing sustained insulin independence[19].

5.4.2 EMBRYONIC STEM CELL TREATMENT:

Human ESCs, derived from the inner cell mass from embryos fertilized in vitro, give rise to all differentiated cells through a series of self-renewal and differentiation processes. Thus, being pluripotent, they can be differentiated into any definitive cell type, such as the insulin producing ß-cells, on exposure to appropriate signals in the correct sequence over appropriate time periods. However, it is important to establish human ESCs that behave like primary ESCs for unlimited supplies of the cells. Soria and colleagues successfully derived insulin-producing ß-cells from mouse ESCs[19].

However, there are major obstacles with regard to the use of ESCs in therapeutic purposes, such as:

1) *Ethical and Religious Concerns:*Many government bans have been put on the use of ESCs since it requires the destruction of human embryos, which is against the moral rights of the embryo[18].

2) *Establishment and Cell Line Expansion:* Before ESCs can be used in therapeutics, it is essential to obtain and expand the cell lines from human embryos without contamination and loss of their pluripotent properties. Human ESCs have been expanded as undifferentiated colonies on feeder layers of mouse embryonic fibroblasts. Due to this, the limited supplies of human ESCs have been contaminated with mouse genes and certain viruses associated with mice, reducing the potential of the cells in therapeutics. However, it was recently discovered that it is now possible to expand ESC lines without the use of the feeder layer[18].

3) *Development:*The obtained ESC lines have to differentiate into functioning ß-cells in order to improve diabetic conditions. Signalling the neighbouring cells, not destined to develop into ß-cells, is important to influence the signalling of the progenitor cells to differentiate into ß-cells*(Figure-13)*. Certain signalling factors play an important role in the development of ß-cells, but little is known. The extra-cellular matrix proteins also play an important role in the differentiation of islet cells[18].

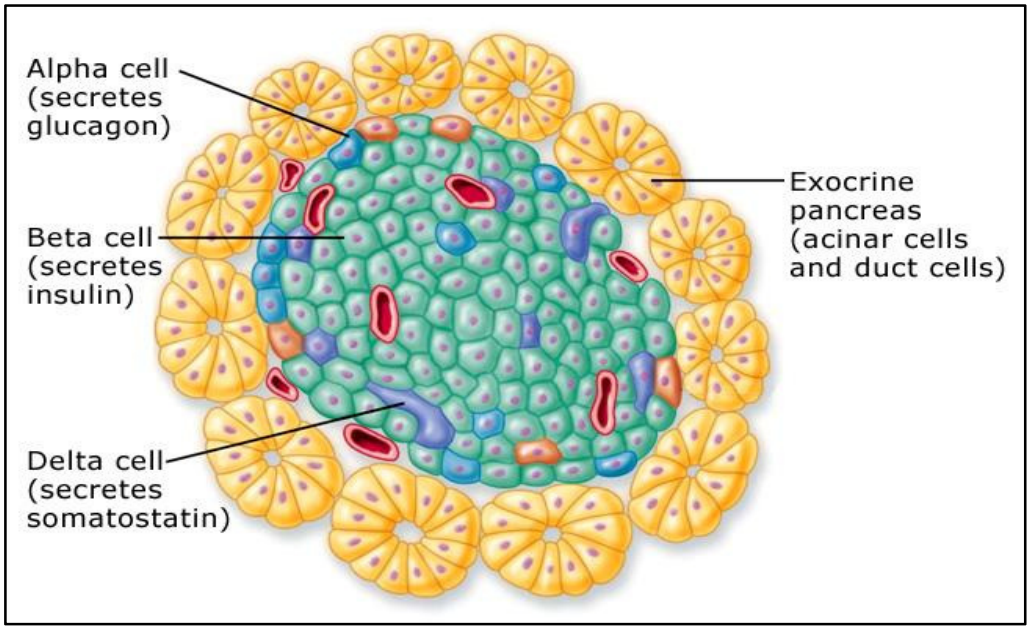

Figure-13. Cross-section of the Islet of Langerhans: The Islet of Langerhans comprise of the endocrine cells (α-cells, ß-cells, and δ-cells) of the pancreas.

(Ref: http://www.wonderwhizkids.com/conceptmaps/Pancreas.html)

4) *Rejection:* The ß-cells differentiated from the ESCs must be protected from rejection by the body in order to avoid the use of immunosuppressant drugs[18].

5) *Target Form:* What is the target form to be acquired by the ß-cells? In the adult pancreas, the ß-cells are present in minimal numbers in the core of the islets, while other cell types (α-, δ- and PP-cells) are abundantly present on the periphery of the islets. The ß-cells are also tightly coupled to each other via gap junctions, allowing direct electrical signalling between neighbouring cells. This structural specificity of the ß-cells is essential for secretion of insulin. Thus, it is a major challenge to reproduce this niche and obtain functional ß-cells[18].

6) *Administration:* Several considerations arise with regard to where the cells or cell clusters have to be transplanted and in what form. ESCs have the potential to develop cancers and other forms of teratoma due to their continuous self-differentiation ability. It has also been observed that intra-portally transplanted ß-cells secrete insulin directly into the liver, rather than into the systemic circulation[18].

7) *Lifespan of the Cells:* The lifespan of the islet/ß-cells is limited. It has been found that patients who received this treatment had to revert to insulin treatment within 5 years. Thus, as an ultimate cure, these cells would have to maintain their proliferative and functional ability, or transplantations would have to be repeated at regular intervals. In order to maintain function, continuous replication is needed, which increases risks of teratoma formation due to loss of cell cycle control[18].

5.4.3 ADULT STEM CELL TREATMENT:

ESCs have various drawbacks to their use in therapeutics for diabetes due to the above discussed factors. As an alternative, ASCs can be used instead. Mesenchymal stromal cells (MSCs) are a major type of ASCs and are the non-hematopoietic stem cells present in the bone marrow. They are generally involved in tissue regeneration and repair due to production of matrix proteins. They have unique systemic immunomodulatory properties that reduce the risk of graft-versus-host diseases and the utilization of immunosuppressants. They are capable of suppressing T-cell proliferation in response to alloantigens and upregulate the number of regulatory T-cells. Such immunosuppression is also associated with reduction in inflammatory cytokines. MSCs can be expanded *ex vivo* and moreover, show no risk of cancerous tumour development and/or ectopic tissue formation[21].

MSCs can be harvested from several organs and not only the bone marrow. Although MSCs are capable of immunomodulation, cells of autologous origins are preferred to avoid risks of infections or possible rejection. Thus, autologous MSC treatment for Type-1 diabetes was considered as a safer and feasible strategy[21].

6.0 CONCLUSION

It was evident that stem cells, without doubt, have the potential to treat many human afflictions, including cancer, diabetes and neurodegeneration. Although it is interesting to work with stem cells, the overriding concerns for any newly developed stem-cell-based treatment remain the same, *i.e.*, efficacy, safety and affordability.

There are many human ESC lines already in existence and banking of clinical-grade cells is underway, enabling optimal immunological matching of donors and patients. The differentiation potential of ASCs is well-characterized *in vivo* (HSCs) and *in vitro* (MSCs), but, the trans-differentiation potential of most ASCs remains controversial, partly as a consequence of culture conditions, contaminations and cell-fusion events. ASCs, ESCs, and iPSCs can be used in drug discovery, to screen for compounds stimulating self-renewal or promote specific differentiation programmes. CSCs are responsible initiation, development and metastasis of tumours, and also attribute to therapeutic resistance. Discovery and design of drugs that selectively target CSCs offers the potential to develop cancer-specific treatments that are not only more effective, but also cause less damage to the patient's tissues than the drugs currently in use. An alternative strategy to stem cell transplantation is to stimulate the patient's own endogenous cells to divide or differentiate *in vivo*, as seen to occur during natural skin wound healing.

TE is an application of stem cells that combines the fields of cell transplantation, materials science, and engineering. A range of biomaterials are already in clinical use for tissue repair and regenerative medicine, for example, to repair defects in cartilage and bone. Biodegradable or resorbable scaffolds are exploited for delivery and release of small molecules, growth factors and peptides. These scaffolds are made from different types of biomaterials, depending on their applications. Stem cells are intricately connected with their surroundings and constantly receive inputs from their niches, which direct their subsequent behaviour. By targeting dysfunctional and deregulated niches, niche-directed therapies are designed to combat stem cell loss, such as that which occurs in response to organismal ageing and degenerative disease, and to prevent of reverse malignant transformation for the treatment of hematopoietic and non-hematopoietic tumours.

Diabetes Mellitus is characterized by hyperglycaemia, either due to lack of sufficient insulin or impaired secretion of insulin, caused by defects in the ß-cell population of the Islets of Langerhans in the pancreas. This indicated that the ß-cell mass is important for maintenance of normoglycaemia. Potential therapy for diabetic individuals could thus be, stem-cell-based treatments such as use of either ESCs, or ASCs to grow pancreatic ß-cells in vitro, followed by transplantation into the diabetic patient.

As an important field in the golden era for developmental biology, a characteristic feature of stem cell research is the motivation to benefit human health. It provides the opportunity for interdisciplinary research, as it brings together biologists, clinicians, and researchers across the fields of physical sciences and mathematics. It also fosters partnerships between academics, and the biotechnological and pharmaceutical industries.

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