

Advances in stem cell therapy

- An overview

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Introduction

A stem cell is essentially the building block of the human body. Unlike a regular cell, which can only replicate to create more of its own kind of cell, a stem cell has the capability to renew itself for longer periods of time or become a specialized cell. Stem cell research and therapy is a promising advancement in medical science. The significance of stem cell therapy in modern medicine is that it offers hope in the treatment of incurable diseases like spinal cord injuries, diabetes, Alzheimer's disease, multiple sclerosis, aids, Parkinson's disease etc. The process of stem cell treatment is relatively simple. Stem cells are extracted from an embryo, an umbilical cord, blood or even bone marrow. They are then replicated in a cell culture. These new cells are then transplanted to the problem area in the patient to help repair damaged tissue. A successful stem cell therapy requires an understanding of how stem cells work, combined with a reliable approach to ensure that the stem cells perform the desired action in the body. In this article, we reveal briefly how stem cells work, discover their potential to treat disease and get inside the fierce debate surrounding their research and use.

Key-words: Stem cells, Plasticity, Types, Transplantation, Therapy, Advantages.

Stem cells are the cells from which we are made. An understanding of the processes that regulate the growth and differentiation of stem cells is necessary for their use in the treatment of several diseases. Plasticity, homing and engrafting are the properties of stem cells by making use of which the treatments have been designed. The pathology of a disease and the role of stem cells in treating the disease must be well established. Medical researchers believe that stem cell therapy has the potential to dramatically change the treatment of human disease. The types of transplants in stem cell therapy, mechanism of action of stem cells and their role in treating various diseases have been discussed.

Stem Cells

We are aware that different types of cells make up our body all of these different cell types arose from a single cell, called the zygote. It has no particular function and divides forming two, four and eight cells which are totipotent having ability to differentiate into embryonic and extra embryonic cell types; each of these cells divide again and again resulting in a hollow ball like structure called blastocyst that contains two types of cells, the trophoblast and inner cell mass^[1].

There are two types of stem cells.^[2]

Embryonic stem cells (ESC): They are obtained from the inner cell mass which are pluripotent cells and can become any tissue in the body, excluding placenta. ESC have both the capacity to self-renew, thus maintaining a continuous supply of stem cells and the ability to give rise to specialized cell types, such as liver cells or brain cells i.e., unspecialized stem cells give rise to specialized cells called differentiation. Scientists just begun to understand the signals inside and outside cells that trigger stem cell differentiation. The internal signals are controlled by a cell's genes, which are interspersed across long strands of DNA, and carry coded instructions for all the structures and functions of a cell. The external signals include chemicals secreted by other Adult stem cells typically generate the cell types of the tissue in which they reside. Once differentiated, cells remain so and usually lose their ability to divide. Specifically, ESC are derived from embryos that develop from eggs that have been fertilized *in vitro* and then donated for research purposes and therapy with informed consent of the donors. Human ESC grow as homogeneous and undifferentiated colonies when they are propagated on a feeder layer of mouse embryonic fibroblasts (MEFs). They have a normal karyotype and express telomerase and embryonic cell-surface markers. Removal from the MEF feeder layer is associated with differentiation.

Adult stem cells: They are undifferentiated cells found among differentiated cells in a tissue or organ (the origin of which in mature tissues is unknown) that can renew and can differentiate to yield the major specialized cell types of the tissue or organ. The primary roles of these cells in a living organism are to maintain and repair the tissue in which they are found. They have been identified in many organs and tissues but they are very small number in each tissue. They are thought to reside in a specific area of each tissue where they may remain quiescent for many years until they are activated by disease or tissue injury.

Normal differentiation pathways of adult stem cells:

- Bone marrow contains two kinds of stem cells. Hematopoietic stem cells that give rise to all the types of blood cells. Bone marrow stromal cells (mesenchymal stem cells) that give rise to osteocytes, chondrocytes, adipocytes and other kinds of connective tissue cells such as those in tendons.
- Neural stem cells in the brain give rise to neurons, astrocytes and oligodendrocytes.
- Epithelial stem give rise to absorptive cells, goblet cells, and enteroendocrine cells.
- ⊗ Skin stem cells give rise to keratinocytes. The follicular stem cells can give rise to both the hair follicle and to the epidermis.

Adult stem cell plasticity and transdifferentiation:

Adult stem cells were thought to be restricted to produce differentiated cells, which were specific to the organ from which they were isolated. Recently, several examples have been reported which demonstrate that these stem cells, under certain conditions, can be induced to form other cell types (transdifferentiation)^[3]. For example:

Hematopoietic stem cells may differentiate into: skeletal muscle cells; cardiac muscle cells; three major types of brain cells and liver cells. Bone marrow stromal cells may differentiate into: cardiac muscle cells and skeletal muscle cells. Brain stem cells may differentiate into: blood cells and skeletal muscle cells.

Relative technical merits of embryonic and adult stem cells:

Adult and embryonic stem cells have different characteristics, and any therapeutic applications developed from them would have different strengths and weaknesses^[4].

Embryonic stem cells are capable of generating all the cell types of the body. They can be easily isolated, multiplied, and maintained in culture but they prone to trigger the development of tumors, usually benign, known as teratomas. Adult stem cells may be limited to differentiating into the different cell types that exist in the tissue from which they were obtained, though there is some evidence that adult stem cells may be less limited than previously believed. They are difficult to isolate, multiply, and maintain in culture. Adult stem cells derived from a patient's own body can be used for therapeutic purposes without fear of immune rejection. Therapeutic use of adult stem cells may require the use of SCNT (Somatic Cell Nuclear Transfer) or the development of other new techniques to avoid immune rejection.

Sources Of Stem Cells

Stem cells can come from leftover embryos stored at fertility clinics (Spare embryos), Cloned embryos, Umbilical cord cells, Aborted fetuses, Adult tissue or organs, Cadavers (isolation and survival of neural progenitor cells from human post-mortem tissues (up to 20 hours after death) has been reported).

Types Of Stem Cell Transplants:

There are many types of stem cell transplants. This section defines each of the various types of transplants^[6-10].

Based on the source of the stem cells

Peripheral blood stem cell (PBSC) transplants: They are obtained from the peripheral blood. PBSC transplants are performed more often because they are easier to collect, provide a more reliable number of stem cells, the procedure puts less strain on the donor's system, and the patient recovers more quickly.

Bone marrow transplants: They are obtained from the bone marrow. Bone marrow transplants are sometimes used if insufficient numbers of stem cells can be obtained from the peripheral blood.

Cord blood transplants: They refer to transplants where the stem cells are obtained from umbilical cord blood. Historically they have not been used frequently due to limited numbers of stem cells that can be collected from each umbilical cord. Recently, exciting new data have been generated using multiple cord blood units from more than one donor.

Based on the donor who provides the stem cells

Autologous stem cell transplants (autografts): In this type of transplant, patient's own stem cells from either bone marrow or

circulating blood are removed before treatment and then frozen. After getting high doses of chemo and/or radiation the stem cells are thawed and given back to you. An advantage of this transplant is that patient gets own cells back without risk that immune system will reject the transplant. A possible disadvantage is if donor has cancer, cancer cells may be harvested along with the stem cells and then put back into your body. To prevent this, doctors may treat stem cells with anti-cancer drugs or other therapies to reduce the number of cancerous cells that may be present. This is called purging. This kind of transplant is mainly used to treat leukemias, lymphomas, and multiple myeloma, but it is sometimes used for other cancers.

Allogeneic stem cell transplants (allografts): Here, the stem cells do not come from the patient, but from a donor whose tissue type best matches the patient. Our immune systems attack things they don't recognize, including cells and tissues. Therefore, the transplanted stem cells must match the recipient closely. To determine whether the donor is a good immunological match with the recipient, a tissue typing test is performed using blood samples from both individuals. This test identifies certain proteins, called HLA antigens, which reside on the surfaces of specific immune cells. If both individuals have identical HLA antigens, they are a good match. The donor is most often a family member, usually a brother or sister. If patient do not have a good match in the family, a donor may be found from the general public through a national registry. Blood taken from the placenta and umbilical cord of newborns is a newer source of stem cells. This small amount of blood has a high number of stem cells. An advantage of this transplant is that the donor stem cells produce their own immune cells, which may help destroy any cancer cells that remain after high-dose treatment. Another advantage is that the donor can often be asked to donate more stem cells if needed. Stem cells from healthy donors are also free of cancer cells. Still, there are many possible drawbacks to allogeneic stem cell transplant. The transplant, also known as a graft, may not "take" that is, the donor cells may be more likely to die or be destroyed by the patient's immune system before settling in the bone marrow. Another possibility is that the donor cells will make new immune cells that attack the recipient's body - a condition known as graft-versus-host disease. There is also small risk of certain infections from the donor cells, although donors are always tested beforehand to minimize this risk. This type of transplant is most often used to treat leukemias, lymphomas, and other bone marrow disorders. They are usually performed in the context of clinical trials.

Syngeneic stem cell transplants: This is a special kind of allogeneic transplant because the donor is an identical twin with identical tissue types. An advantage is that graft-versus-host disease will not be a problem and disadvantage is this type of transplant won't help destroy any remaining cancer cells in cancer. So every effort must be made to destroy all the cancer cells before the transplant is done.

Tandem autologous transplant: It is a "double autologous transplant". In a tandem transplant, a patient gets 2 courses of high-dose chemo, each followed by a stem cell transplant. All of the stem cells needed are collected before the first high-dose chemo treatment and half of them are used for each procedure. Most often both courses are given within 6-months, with the second one done after the patient recovers from the first one. Researchers hope that this method can keep the cancer from coming back and are still studying how this method can best be used. This type is being used for the treatment of certain types of cancer, including multiple myeloma, Hodgkin disease, and non-Hodgkin's lymphoma.

Mini (nonmyeloablative) allogeneic transplant: Another type of allogeneic transplant is called a reduced-intensity transplant, non-myeloablative transplant, or mini-transplant. This transplant uses less intense chemo and/or radiation to get the patient ready for the transplant

compared with a standard allogeneic transplant. In this procedure, the patient is given low doses of chemo not enough to destroy all the cancer or all of the bone marrow, but enough to suppress the patient's immune system. The new immune cells then begin to destroy the remaining cancer cells, in what is known as a "graft-versus-tumor" effect. Unlike the standard allogeneic transplant, cells from both the donor and the patient may exist together in the patient's body for some time after a mini-transplant. But slowly, over the course of months, the donor cells take over the bone marrow and replace the patient's own bone marrow cells. These new cells then develop an immune reaction to the cancer and kill off the patient's cancer cells. The advantage of a mini-transplant is high doses of chemo and/or radiation is not needed. This makes it useful in older patients, those with other health problems who aren't strong enough for a normal stem cell transplant, or patients who have already had a transplant. Mini-transplants have been found to treat some diseases better than others. They may not work well for patients with a lot of disease in their body at the time of transplant or those with fast-growing disease. Also, the lowered immune response could still lead to graft-versus-host disease. Ways to improve the procedure are still being studied. Another possibility that is being studied is autologous transplant followed by non-myeloablative allogeneic transplant. This decreases the amount of cancer.

Collection Of Stem Cells

Most of the stem cells in the human body reside in the bone marrow. Until recently, the only way to obtain stem cells for transplantation was to remove a portion of the bone marrow^[11-19]. However, through recent medical advances, it is now possible to collect stem cells from a person's peripheral blood.

Collection from bone marrow:

Collecting or "harvesting" bone marrow is usually done in a hospital operating room under general anesthesia. Surgeon will take bone marrow from several different areas of the hip bone (pelvis). The bone marrow is frozen and stored until high-dose chemotherapy is completed.

Collection from peripheral blood:

Because most stem cells reside in the bone marrow, it is necessary to move them from the bone marrow to the bloodstream. This procedure is called mobilization. A commonly used mobilization technique is to administer a medication called a colony-stimulating factor or "growth factor" which is a cocktail of natural proteins, helping the stem cells to make blood cells more quickly so that it takes less time to recover from the transplant. It is given as injection under the skin, much like an insulin and has no side effects. Some people have itching around the injection site. Some pain in bones after a few injections because of lot of blood cells being made inside the bones may occur. Once a sufficient number of stem cells are mobilized from the bone marrow into the bloodstream, they collected using a non-surgical procedure called apheresis. Apheresis involves removal of whole blood from a patient or donor within an instrument that is essentially designed as a centrifuge, where the components of whole blood are separated. One of the separated portions is then withdrawn and the remaining components are retransfused into the patient or donor.

Cryopreservation

Following collection and processing, the blood or bone marrow (now referred to as the stem cell transplant) must be preserved to keep the stem

cells alive until it is time to infuse the cells into the patient's bloodstream. To preserve, the cells are frozen and stored in liquid nitrogen or a special freezer as long as necessary. A chemical called DMSO (dimethyl sulfoxide) is mixed which keeps the water in the cells from forming ice crystals that would damage the cells during the freezing process. Some side effects are caused by DMSO following infusion, they include nausea and vomiting, abdominal cramping, chilling, an unusual odor, and taste of garlic and other side effects depends organ of treatment.

Mechanism Of Action Of Stem Cells

Stem cells are capable of performing three important functions with unique abilities^[20-23].

Plasticity: Potential to change into other cell types like nerve cells.

Homing: To travel to the site of tissue damage.

Engraftment: To unite with other tissues.

The respective stem cells do not have to be implanted into a damaged organ, they can be implanted into more accessible superficial tissue because they will find their way into the damaged organ, as if 'attracted' by it. This means, if we inject our specific stem cell into a patient who has a nerve disorder, the cell migrate to the site of injury attracted by specific chemicals released by the damaged tissue. The cell, after homing to the damaged area will fuse with the damaged tissue by the process of engraftment and become the same tissue by displaying the property of plasticity. The rest of the injected cells which have not migrated or engrafted will travel to the bone marrow where they will be stored with the body's blood cells until needed. They can still respond, from the bone marrow, to signals from damaged tissue elsewhere in the body and migrate to that site. This is why responses are sometimes not noted until a few months after treatment. Immediately after the cells are injected, the body secretes numerous chemicals called cytokines. They can cause the remarkable effects sometimes seen immediately after treatment, but are usually transitory, although these effects have persisted on occasion.

Stem Cell Therapy For Several Disease / Disorders

Stem cell therapy in myeloma:^[10-18]

Multiple myeloma (also known as myeloma, plasma cell myeloma, or as Kahler's disease) is a cancer of plasma cells. The cells metastasize, often spreading to neighboring bone. These immune cells are formed in bone marrow, are numerous in lymphatics and produce antibodies. Myeloma is part of the broad group of diseases called hematological malignancies. Stem cell transplantation, performed as support for high-dose chemotherapy, is a treatment option for many patients with myeloma. Studies have shown that this treatment improves both the response rate and survival in myeloma over that obtained with conventional chemotherapy. Because high-dose chemotherapy also destroys normal blood-producing stem cells in the bone marrow, these cells must be replaced in order to restore blood cell production.

The first step in the process is the collection of stem cells from a patient or a donor which involves the use of colony stimulating factors (CSFs). Some of the CSFs used in the treatment of myeloma include: Leukine(sargramostim) Granulocyte macrophage CSF (GM-CSF)- Stimulates the production of WBCs known as granulocytes and macrophages. Neupogen (filgrastim) G-CSF- Stimulates the production

of WBCs known as granulocytes. Neulasta (pegfilgrastim) Pegylated G-CSF- long-acting form of filgrastim. After the bone marrow or stem cells are collected, or at some later date, the patient will receive high-dose chemotherapy designed to destroy cancer cells more effectively than standard chemotherapy. Depending on the type of cancer and other factors, some patients may receive one or more treatments of high-dose chemotherapy, possibly in combination with radiation therapy, over a period of several days. This combination of treatments is also referred to as a conditioning regimen. These treatments, in addition to killing cancer cells, also destroy the blood-producing cells in the bone marrow, hence the need for the stem cell transplant. The chemotherapy drug melphalan is the most commonly used conditioning regimen in myeloma. The frozen bags of bone marrow or blood cells are thawed in a warm water bath and infused into a vein over a period of 2 to 4 hours which travel to the bone marrow (engraftment) and begin to produce new blood cells, replacing the normal cells lost during high-dose chemotherapy within 12 to 15 days following infusion. CSFs may be administered during this time to stimulate the process of blood cell production. For the first 2-4 weeks after the transplant (until engraftment is complete) a transplant recipient is susceptible to infection, anemia, and bleeding caused by low blood cell counts which weaken the body's immune system. Therefore, special precautions are necessary during recovery. Patients may be given red blood cell and platelet transfusions during the recovery period to help prevent anemia and bleeding. Antibiotics are often prescribed to help prevent infection. High-dose chemotherapy and stem cell transplantation are typically performed following several cycles of conventional chemotherapy (also known as induction therapy). Induction therapy is performed first in order to reduce the tumor burden. Most of the significant potential side effects are a result of the high-dose chemotherapy. Some of the more common temporary side effects include nausea, vomiting, diarrhea, mouth sores, skin rash, and hair loss.

Stem cell therapy in Parkinson's disease: ^[24-26]

Parkinson's disease is a degenerative disease of the central nervous system that often impairs motor skills, speech, and other functions. The primary symptoms are the results of decreased stimulation of the motor cortex by the basal ganglia, normally caused by the insufficient formation and action of dopamine, which is produced in the dopaminergic neurons of the brain. To replace the dead cells, the researchers needed to find stem cells that could differentiate into dopamine neurons.

Blastocyst embryonic stem cells: Creating pure neuronal cells from embryonic stem cells (ES) had been problematic as the cells did not always differentiate into neurons. Sometimes they became glial cells, which lack many of the neurons desirable properties. Even when the neuronal cells were created successfully, they often died in the brain following transplant, a process called programmed cell death or apoptosis. In addition, the cells would sometimes become tumors. By inducing ES cells to express a protein called myocyte enhancer factor 2C (MEF2C) the problem has been solved. MEF2C is a transcription factor that turns on specific genes which then drive stem cells to become nerve cells. Using MEF2C, colonies of pure neuronal progenitor cells with no tumors was created and transplanted into the brain which later became adult nerve cells. MEF2C also protected the cells from apoptosis once inside the brain. The tissue needed for the treatment came from prematurely terminated human fetuses or late-stage embryos. This raised a valid ethical issue for consideration.

Umbilical cord blood stem cells: The multipotent stem cells from umbilical cord blood have the potential to turn into many different types of cells, but their natural fate is to become blood and immune cells.

Adult stem cells: There are many different types of multipotent adult stem cells, each of which is responsible for developing into the cells of a certain type of tissue. The best adult stem cell candidates for the Parkinson's disease treatment would be those that can differentiate into dopamine neurons. Also, at the time this treatment was being developed, however, researchers knew little about adult stem cells and umbilical cord cells. Thus, they were not an option for the treatment.

To treat Parkinson Disease they applied a unique procedure, specifically designed for Parkinson's patients, in which adult stem cells extracted from the human retina, called human Retinal Pigment Epithelial cells (hRPE) were implanted through brain injection. These special cells have the quality of producing Dopamine which gives solution to the lack of Dopamine production, they have no immunosuppressive reactions and therefore patients do not need to take additional drugs during the treatment. hRPE cells are implanted in the brain where the damaged cells are, along with a daily cocktail of medications that fertilize this area, helping the cells to survive and keep on renewing themselves. While the treatment can not completely remove all symptoms of the disease, it can greatly reduce the tremors, decrease muscle tension, improve movement's continuity and balance, increase the muscle strength, reduce the freeze ups, etc. The hRPE cells will be injected through Serotaxios surgery into the Putman area in the brain. They used that technique since it has the highest accuracy. A single injection of hRPE stem cells will be given to the patient.

Self Stem Cells Activation and Proliferation: Under non-invasive circumstances, through daily IV of individually customized cocktail of neurotrophic medicines they are activating the patients own stem cells and thus turning these cells into nerve precursor cells. These have signalization and migrate to lesions along the nerve developing-chord. Through this procedure they will create a most active and nerve environment in the brain, to allow the new implanted cells to survive, and to start a continuous process of renewing themselves.

Side effects: Both of the procedures can cause minor side effects for 2 to 3 days after the operation such as fever, headaches, dizziness and fatigue. These side effects are expected and should not alarm or concern the patient. Many patients do not suffer any side effects.

Stem cell therapy in heart diseases: ^[2, 27]

The research is likely to put an end to the use of heart transplant. In a cardiac arrest, heart muscle cells die but existing treatments do not repair the damage to the muscle and have a limited effect on heart function. Researches discovered a way to rejuvenate hearts using a specialist version of heart stem cells - known as "progenitor" cells found in small quantities in human hearts. They are thought just there to help the heart recover from normal wear and tear. In healthy people, reduced oxygen supply can occur in certain situations e.g. after an injury. The affected tissues release chemical messengers that 'call' to a type of circulating stem cells (Endothelial Progenitor Cells-EPCs) for help to re-establish blood supply via the growth of new blood vessels. Kinins, for long time considered inflammatory substances, are among the messengers supporting blood vessel growth. It was found that EPCs respond to kinins by travelling to the target tissue and invading it to assist healing. In patients with angina, EPCs cannot respond to the distress call because they lack a kinin sensor (the 'kinin receptor') on their surface. The oxygen-starved tissue is therefore left with reduced blood supply. In heart attack patients they saw that a proportion of the circulating EPCs were able to sense the kinin signal and respond. The findings showed that heart attack patients possess the functional cells needed to repair blood supply to their heart, but they're hidden amongst a muddle of others.

They used kinin like a magnet to attract and extract the most effective repair cells from the mass of different types. This enriched sample should increase the therapeutic potential, especially in heart attack patients where quick and efficient treatment is crucial for long term outcome. A number of stem cell types, including embryonic stem (ES) cells, cardiac stem cells that naturally reside within the heart, myoblasts, adult bone marrow-derived cells including mesenchymal cells, endothelial progenitor cells, and umbilical cord blood cells, have been investigated as possible sources for regenerating damaged heart tissue. All have been explored in mouse or rat models, and some have been tested in larger animal models, such as pigs. A few small studies have also been carried out in humans, usually in patients who are undergoing open-heart surgery. Several of these have demonstrated that stem cells that are injected into the circulation or directly into the injured heart tissue appear to improve cardiac function and/or induce the formation of new capillaries. The mechanism for this repair remains controversial, and the stem cells likely regenerate heart tissue through several pathways. However, the stem cell populations that have been tested in these experiments vary widely, as do the conditions of their purification and application. Although much more research is needed to assess the safety and improve the efficacy of this approach, these preliminary clinical experiments show how stem cells may one day be used to repair damaged heart tissue, thereby reducing the burden of cardiovascular disease.

Stem cell therapy in AIDS:^[28-30]

HIV infection, attacks white blood cells known as T-lymphocytes, which play a central role in the immune system by fighting against infection. The number of T-lymphocytes in the body decreases as the virus spreads and the immune system stops working, leading to the condition known as Auto-Immune Deficiency, or Aids, meaning patients are no longer able to fight off infections themselves. Most Aids patients die from pneumonia or cancers such as lymphoma. Bone marrow contains stem cells that are capable of forming all types of blood cells including the white blood cells that form part of the immune system. Hematopoietic stem cells (HSCs) are one of the few cell types able to resist infection with HIV-1 despite expressing the cell surface molecules to which HIV-1 binds before entering a cell. HSC expression of a protein known as p21Waf1/Cip1/Sdi1 (p21) is required for HSCs to be resistant to infection with HIV-1. HSCs in which expression of p21 was decreased were more susceptible to infection with HIV-1 than cells expressing normal levels of p21. Further analysis showed that p21 did not inhibit HIV-1 entering the cells, rather it prevented the viral DNA integrating into the host cell genome by binding to the HIV-1 integrase complex and preventing it from mediating chromosomal integration. This protective mechanism was specific for HIV-1, as decreased expression of p21 in HSCs did not allow a related virus (SIVmac-251) to productively infect the HSCs. A growth factor that stimulates bone marrow stem cells to enter the bloodstream is injected. Blood is drawn, and the patients' own stem cells are isolated. Genes which curb the spread of HIV inside the body are isolated, introduced them into human stem cells in a laboratory using a harmless modified virus related to HIV. Stem cells are then returned to the bloodstream, where they make all the different types of blood cells, each of which will have the new anti-HIV gene. Small quantities of the transplanted stem cells were able to grow and produce new white blood cells resistant to HIV, resulting in an improvement in the patients' conditions. The technique involves isolating genes which curb the spread of HIV inside the body, introducing the genes into human stem cells in a laboratory, then transplanting the stem cells into a patient's bone marrow. Small quantities of the transplanted stem cells were able to grow and produce new white blood cells resistant to HIV, resulting in an

improvement in the patients' conditions. The method can initially protect about 10 percent of patients' stem cells, but this percentage increases as HIV kills the vulnerable cells while protected cells replicate. To accelerate the process, six months later the patients stop taking anti-virals for four weeks to give HIV the opportunity to kill some unprotected blood cells; this puts pressure on the protected cells to replicate more quickly and replace the killed cells. Twelve weeks later, the patients go off medication for at least eight weeks, or longer, depending on how well the strategy is working.

Stem cell therapy in Diabetes:^[31-41]

Diabetes mellitus is a chronic metabolic syndrome characterized by increased levels of blood glucose, referred to as hyperglycemia. Type 1 diabetes generally results from autoimmune destruction of pancreatic islet b-cells. Type 2 diabetes is associated with insulin resistance. The current treatment of insulin does not represent a cure because insulin dosage is difficult to adjust. Exogenous insulin frequently fails to achieve optimal glucose control even when intensive regimens are used and pancreatic transplantation involves a surgical procedure and immune rejection problems so, they had gone for transplanting islets which carry a lower antigenic property than the whole organ. Islets can also be manipulated in vitro before transplantation to reduce graft rejection. The disadvantage is it is costlier and requires at least two donor organs for each recipient (Edmonton protocol which combined steroid-free immunosuppression with at least two separate islet infusions to deliver a sufficient total islet mass to the liver to achieve insulin independence had shown marked improvement of islet graft survival) and therefore its broad application remains limited due to lack of sufficient cadaveric donors.

Alternate sources of insulin-producing cells: Alternative cell sources to cadaveric islets, such as stem cells hold great promise to provide a ready source of transplantable, insulin-secreting tissues that would not be limited by the supply of donor organs. In developing a potential therapy for patients with diabetes, researchers hope to develop a system that meets several criteria. For diabetes therapy, it has been observed that the presence of all the islet cell types is preferable to only beta cells since the former are better able to respond to changing levels of glucose in the blood. Therefore, it is important to develop a system in which stem or precursor cell types can be cultured to produce all the cells of the islet cluster in order to generate a population of cells that will be able to coordinate the release of appropriate amount of insulin to physiologically relevant concentrations of glucose in the blood. The main goal is not only induction of insulin biosynthesis, but also its correct processing, storage and regulated secretion in response to physiologic signals, without which such cell-therapy approaches would not be significantly advantageous over insulin administration.

Adult pancreatic stem cells are located in intra islet, nestin-positive cells, duct cells and oval cells which differentiate into pancreatic b-cells. Advantage is that they behave as an autologous model. The benefits of the Embryonic Stem Cells include the possibility of propagating an unlimited number of cells that possess the ability to become fully functioning endocrine tissue. Other stem cell sources include Pancreas, Pancreatic duct, Foetal tissues, Non-pancreatic stem cells (hepatocytes, enterocytes, bone marrow, cord blood stem cells, etc.). In contrast to mesodermally derived cardiomyocytes and ectodermally derived neural cells, endoderm-derived cells, including b-cells do not differentiate spontaneously from in vitro culture of stem cells. Consequently, the embryonic stem cells require supplementary and specialized transcription factors to be directed into a controlled pathway of

endocrine development. Several approaches have been evaluated to know the transcriptional factors that regulate endocrine and exocrine differentiation in establishing endocrine lineage. In normal pancreatic development, down regulation of Sonic hedgehog (Shh) protein is a key event in the induction of Pdx-1 expression, which in turn programs for the development of pancreatic progenitor cells. Pancreatic differentiation begins in cells expressing Hnf6 that induces the expression of Hnf3b, which is an essential factor in endodermal cell lineage development. Neurogenin3 (Ngn3) is a 'helix-loop-helix' transcription factor that plays a central role in endocrine pancreas neogenesis from embryonic gut endoderm. Activation of signalling factors from Ngn3, such as 'delta' and 'notch' decides the differentiation of cells into endocrine and exocrine origins respectively.

Stem cell therapy in lung diseases:^[42-55]

Lung cancer characterized by uncontrolled cell growth in lung tissues and idiopathic pulmonary fibrosis (IPF) also known as Lung Fibrosis, characterized by the scarring of the lung tissue in which the Alveoli (air sacs) become inflamed and develop scars on the lung tissue in an attempt to repair itself as a result the lung tissue is gradually replaced by fibrous tissue, which prevents the alveoli sacs from inhaling oxygen. The lung tissues harden causing shortness of breath, chronic dry cough, and discomfort in the chest. These are the two conditions for which new treatments are desperately needed. Initially the focus of research was to harness the potential of bone marrow stem cell plasticity to repair damaged organs by differentiating into epithelial cells to replace the injured areas as adult organs have limited regenerative capacity. More recently it has become clear that engraftment of these stem cells as epithelial tissue is a rare event with perhaps limited clinical significance, But can incorporate into various tissues and in some cases take on characteristics of the tissue to which they have homed suggesting their role as a treatment modality either by modulating their role in disease or by using them as a vector for treatment delivery. The ability of bone marrow derived cells to specifically home to a wide range of pathological conditions such as organ fibrosis and tumors and then to incorporate into these areas suggest that they may be perfect vectors to deliver anti-fibrotic or oncological therapies. As a subgroup of the adult bone marrow stem cells, Mesenchymal stem cells (MSC) have several properties in addition to their homing capabilities that incline them toward a role as a vector. MSCs can be relatively easily transduced and expanded in culture for many passages, while retaining their growth and multi-lineage potential. They also seem to be relatively immunoprivileged due to their expression of major histocompatibility complex (MHC)1, but lack of MHC2, and the co stimulatory molecules CD80, CD86, CD40. This property may allow the delivery of allogeneic MSCs without prior immunomodulation. BMSCs in particular are able to produce differentiated cells not restricted to their lineage.

Previous studies had an evidence for contribution of bone marrow derived cells to areas of both physiological and pathological extracellular matrix deposition including wound healing, tissue stroma, and organ fibrosis. The bone marrow recruitment to areas of pathological fibrosis and wound healing may provide novel ways for treating these disorders. Contrary to acting solely as a supporting structure, tumor stroma is integral to the behavior of the tumor, including cancer spread and growth. It is composed of fibroblasts and myofibroblasts, which produce extracellular matrix and the "desmoplastic reaction," including endothelial cells involved in angiogenesis, and inflammatory cells. Myofibroblasts in the tumor stroma secrete growth factors and proteolytic enzymes that influence tumor invasion and progression. In some situations the presence of a tumor capsule has been shown to be

protective, leading for example to improved prognosis in human hepatocellular carcinoma. Conversely, increased stroma and myofibroblast numbers have been associated with a worse prognosis, with the proliferative activity of stromal fibroblasts correlated to breast cancer metastasis. As with repair and fibrosis, bone marrow derived stem cells contribute to a desmoplastic response in the form of myofibroblasts and fibroblasts. Tumor neovasculation is one of the hallmarks of cancer, and a contribution of bone marrow derived stem cells to the angiogenesis of tumors has also been demonstrated. Bone marrow cells (Sca⁺) labeled and injected intravenously were shown to incorporate as endothelial-like cells into the periphery of a glioma. In contrast, other studies have only shown a minimal contribution of bone marrow cells to the newly formed tumor endothelium. Mesenchymal stem cells alone have been shown to have an ability to specifically target tumor tissue. *In vitro* migration studies have demonstrated an enhanced migration of MSCs toward tumor cells, suggesting the role of soluble chemokines. Possible candidates include platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and stromal cell derived factor 1 (SDF1), which have all demonstrated enhanced MSC migration *in vitro*. Homing of adult hematopoietic stem cells to injured tissue involves chemokine ligands and receptors in a similar fashion to the recruitment of leukocytes to areas of inflammation. The importance of the chemokine CXCL12 (SDF-1) and its receptor CXCR4 has been well established for hematopoietic stem cells. It is important to note that the ability of MSCs to home and migrate appears to decrease during *in vitro* expansion in relation to their loss of surface expression of chemokine receptors. Increased CXCL12 (SDF-1) levels and numbers of cells expressing CXCR4 have been shown in lung tissue samples of patients with idiopathic pulmonary fibrosis. *In vitro*, the migration of MSCs toward lung lysates was blocked by a CXCR4 antagonist that was also able to reduce the amount of fibrosis *in vivo*. A similar model of lung fibrosis was used to show that the number of bone marrow derived fibrocytes in the injured lung and the resulting fibrosis could be reduced by the inhibition of CXCL12. This study also, however, suggested the importance of other chemokine/receptor combinations, while in other studies secondary lymphoid chemokine (SLC)/CCR7 and CCR2 have been implicated in bone marrow cell homing to mouse models of lung fibrosis. One *in vitro* study examined the differences in gene expression profiles between MSCs exposed to conditioned medium from tumor cells and bone marrow cells. It appeared that the CXCL12 (SDF-1)/CXCR4 axis was important, but that the MSCs produced the chemokine, which then acted in an autocrine manner.

The use of exogenous MSCs as vectors for targeted delivery of therapies and genes in tumors and areas of fibrosis is promising however, it is important to consider the roles of these cells in disease beyond their function as an inert vehicle. The contribution of bone marrow derived stem cells to the pathogenesis of organ fibrosis is equally as confused. A reduction in the recruitment of these bone marrow cells to areas of fibrosis by the removal of the chemotactic gradient demonstrated a reduction in the amount of fibrosis. Conversely, suppression of the bone marrow with busulphan led to a worsening in mice subjected to such insults, while systemic MSCs appear able to alleviate lung fibrosis. Further studies examining precise cell types and chemotactic factors are imperative to dissect these issues. Conceivably, if bone marrow cell engraftment was high enough, this approach could be used for the treatment of inherited lung diseases such as cystic fibrosis and 1 - antitrypsin deficiency. Alternatively, allogeneic bone marrow cells or genetically manipulated autologous cells can "replace" mutant genes in genetic deficiencies, and animal and small clinical studies have shown potential with published data in osteogenesis imperfecta and lysosomal

storage diseases.

Advantages Of Stem Cell Research ^[56]

- It provides medical benefits in the fields of therapeutic cloning and regenerative medicine, great potential for discovering treatments and cures to a plethora of diseases.
- Limbs and organs could be grown in a lab from stem cells and then used in transplants or to help treat illnesses.
- It will help scientists to learn about human growth and cell development.
- Scientists and doctors will be able to test millions of potential drugs and medicine, without the use of animals or human testers. This necessitates a process of simulating the effect the drug has on a specific population of cells and tell if the drug is useful or has any problems.
- Stem cell research also benefits the study of development stages that cannot be studied directly in a human embryo, which sometimes are linked with major clinical consequences such as birth defects, pregnancy loss and infertility.
- A more comprehensive understanding of normal development will ultimately allow the prevention or treatment of abnormal human development.
- Another advantage of stem cell research is that it holds the key to reversing the effects of aging and prolonging our lives.

Disadvantages Of Stem Cell Research ^[56]

- The use of embryonic stem cells for research involves the destruction of blastocysts formed from laboratory-fertilized human eggs. For those people who believe that life begins at conception, the blastocyst is a human life and to destroy it is immoral and unacceptable.
- Like any other new technology, it is also completely unknown what the long term effects of such an interference with nature could materialize.
- According to a new research stem cell therapy was used on heart disease patients. It was found that it can make their coronary arteries become narrower.

Conclusion

Stem cell therapy is advancement in medical research. However, more basic research remains before stem-cell based therapy is widely used. A potential advantage of stem cells, both embryonic and adult is that, in theory, they could be engineered to express the appropriate genes that would allow them to escape or reduce detection by the immune system. In future stem cells obtained by somatic cell nuclear transfer method may be used for research and therapy to overcome the immune rejection problems and ethical controversies in using embryonic stem cells and difficulty in isolating adult stem cells. The molecular mechanisms of cellular self-renewal must be understood more deeply so that we can efficiently maintain human stem cell lines in their pluripotent state. In addition, the culture methods should be improved to generate sufficient

cells for clinical use. By rectifying some drawbacks and realizing the problem, we can assure that in future, stem cell therapy will be the leading therapy for several diseases.

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