STEM CELLS: A BRIEF HISTORY, CURRENT SCENARIO AND FUTURE PROSPECTS

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Summary

Stem cells are the unspecialized cells that have the capability of giving rise to specialized cells. They are the basis for all cells and are expected one day to cure a wide range of ailments. Embryonic stem cells can evolve into any type of cell in the human body. Adult stem cells come from many parts of the body including umbilical-cord blood, blood vessels, bone marrow, skeletal muscle, brain, skin and liver. Umbilical cord blood stem cells have already been effectively used in the treatment of sickle cell, leukemia, non-Hodgkin's lymphoma, other forms of cancer, life threatening anemia, and autoimmune diseases. Stem cells may hold the key to replacing cells lost in many devastating diseases like Parkinson's disease, Spinal cord injury, multiple sclerosis, Alzheimer's disease, diabetes, chronic heart disease, end-stage kidney disease, liver failure and cancer. Lately stem cell use has been tried in the treatment of burns, infertility, lupus and deafness.

Key Words: Stem cells, unipotent, multipotent, pluripotent, totipotent.

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Introduction

A cell[1,2] is the basic unit of life, sometimes called "the building block of life". It is one of the several characteristics that differentiate the living from the non-living. The process, by which cells change in structure and become specialized in function, is called cellular differentiation. In this process, cells acquire a "type". The morphology of cells may change dramatically during differentiation, but the genetic material (such as DNA within the nuclei) is generally unchanged.

The mammalian cells are of three basic categories:
•Germ cells
•Somatic cells
•Stem cells

Germ cells are the haploid, reproductive cells of the body, such as the gametes. The eggs of a female and the sperm of a male are gametes. **Somatic cells** are diploid cells that make up the human body, such as the skin cells and muscle cells. **Stem cells** are those unspecialized cells that have the capability of giving rise to specialized cells. For instance, blood stem cells give rise to red blood cells, white blood cells, and platelets. Stem cells are primal cells found in all multi-cellular organisms. They retain the ability to renew them through mitotic cell division and can differentiate into a diverse range of specialized cell types. Stem cells possesses two properties,[1,2] viz., self-renewal - the ability to go through numerous cycles of cell division while maintaining the undifferentiated state and unlimited potency - the capacity to differentiate into any mature cell type.

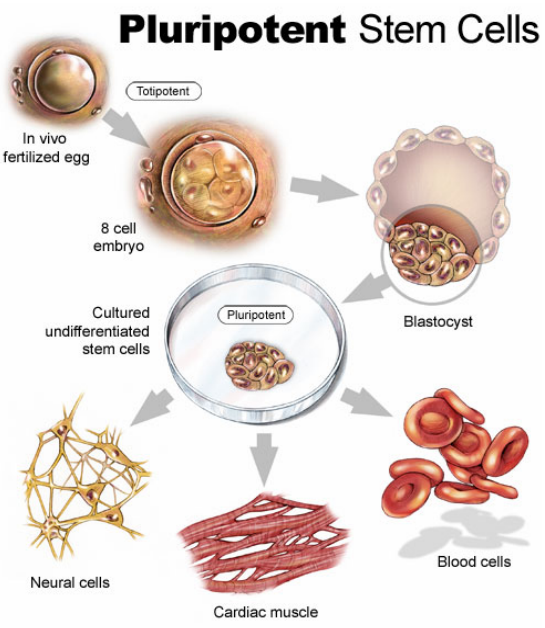
Timeline of Stem Cells

- 1667: Discovery of cell
- 1800: Concept of Inheritance
- 1956: First Bone Marrow Transplant
- 1963: First quantitative description of the self-renewing activities of transplanted mouse bone marrow cells
- 1981: Stem cells discovered
- 1996: First sheep cloned dolly
- 1998: Successful removal of cells from the spare embryos at fertility clinics and growing them in the laboratory.
- 2001: Embryonic stem cells isolated
  Differentiation Gene identified
- 2002: Embryonic stem cells into organ cells
- 2003: Adult stem cells act differently
- 2004: Human brain has adult stem cells

Types of Stem Cells

Based on their potency:

Unipotent cells[1-4]: can produce only one cell type, but have the property of self-renewal which distinguishes them from non-stem cells. Some researchers consider unipotency a property of stem cells but many others believe that cells must be at least capable of multipotency to be a stem cell. Examples of unipotent cells are the human skin cells.



Multipotent cells: can produce only a closely related family of cells. An example of multipotent cells is haematopoietic cells—blood stem cells that can develop into several types of blood cells such as red blood cells, white blood cells and platelets, but cannot develop into brain cells. Pluripotent cells: can give rise to virtually any type of cell except for cells of the placenta, and some of the tissues of the uterus. The inner cells of the blastocyst are pluripotent.

Totipotent cells: can give rise to any other cell and hence considered to be the "master cells" of the body. They contain all the genetic information needed to create any cell of the body. A zygote is totipotent because it is the initial cell which eventually develops into another human being.

Based on their source:

Embryonic Stem Cells:[5,6] (ES cell lines) are cultures of cells derived from the epiblast tissue of the inner cell mass (ICM) of a blastocyst or earlier morula stage embryos. A blastocyst is an early stage embryo—approximately four to five days old in humans and consisting of 50–150 cells. ES cells are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. In other words, they can develop into each of the more than 200 cell types of the adult body when given sufficient and necessary stimulation for a specific cell type. They do not contribute to the extra-embryonic membranes or the placenta. After twenty years of research, there are no approved treatments or human trials using embryonic stem cells. Their tendency to produce tumors and

malignant carcinomas, cause transplant rejection, and form the wrong kinds of cells are just a few of the hurdles that embryonic stem cell researchers still face. Because of their combined abilities of unlimited expansion and pluripotency, embryonic stem cells remain a theoretically potential source for regenerative medicine and tissue replacement after injury or disease.

Adult stem cells:[5,6]

The term adult stem cell refers to any cell which is found in a developed organism that has two properties: the ability to divide and create another cell like itself and also divide and create a cell more differentiated than itself. Most adult stem cells are lineage-restricted (multipotent) and are generally referred to by their tissue origin (mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, etc.). The use of adult stem cells in research and therapy is not as controversial as embryonic stem cells, because the production of adult stem cells does not require the destruction of an embryo. The major function of adult stem cells is to maintain homeostasis in the body in terms of replacing dead or injured cells with new ones that function correctly. The origin of adult stem cells in mature tissue is not clearly known. It is said that adult stem cells are clonogenic (the cells generate identical copies of themselves, then diffirentiate into mature cells of the tissue in which it resides) and unilineal (cell differentiates into a specific, more mature cell, using one or more intermediate steps). In the adult body, they have been identified in various tissues niches, including bone marrow, brain, liver, and skin, as well as in the circulation.

Cord stem cells[7]

In the late 1980s and early 1990s, physicians began to recognize that blood from the human umbilical cord and placenta was a rich source of hemopoietic stem cells (HSCs). This tissue supports the developing fetus during pregnancy, is delivered along with the baby and, is usually discarded. In recent years, however, the multipotent-stem-cell-rich blood found in the umbilical cord has proven useful in treating the same types of health problems as those treated using bone marrow stem cells and Placental Blood Stem Cells. Umbilical cord blood stem cell transplants are less prone to rejection than either bone marrow or peripheral blood stem cells. This is probably because the cells have not yet developed the features that can be recognized and attacked by the recipient's immune system and as umbilical cord blood lacks well-developed immune cells, there is less chance that the transplanted cells will attack the recipient's body, a problem called graft versus host disease. Both the versatility and availability of umbilical cord blood stem cells makes them a potent resource for transplant therapies.

Sources of Stem Cells[8-10]

Autologous

Sources of the patient's own stem cells (autologous), either the cells from patient's own body or his or her cord blood. For autologous transplants physicians now usually collect

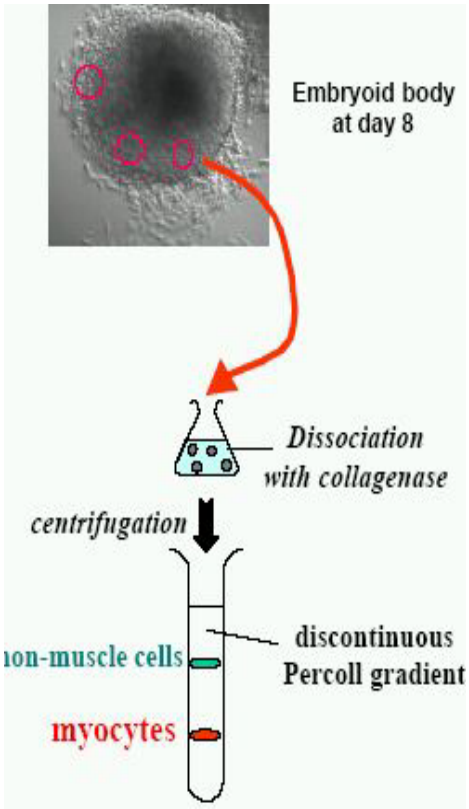
stem cells from the peripheral blood rather than the marrow. A few days before the procedure, donors are generally given a medication (G-CSF [filgrastim], GM-CSF [sargramostim] or a combination of the two to mobilize or force stem cells from the marrow into the circulating blood. These agents can cause flu-like symptoms in the days preceding and following stem cell harvest. To circumvent these two problems, researchers have attempted to isolate adult stem cells from mammalian skin, a highly accessible tissue source.

Allogeneic

Sources of stem cells from another donor (allogeneic), primarily relatives (familial-allogeneic) or completely unrelated donors (unrelated-allogeneic). The stem cells in this situation are extracted from either the donor's body or cord blood. Histocompatibility is a prerequisite for transplantation of allogeneic stem cells. Fetal tissue is the current tissue source for human neural stem cells, raising important ethical issues. Moreover, the use of human fetal tissue, even if acceptable by society, involves heterologous transplantation. Other sources of allogenic adult stem cells include the central nervous system, bone marrow, retina and skeletal muscle.

Xenogeneic

Stem cells from different species are transplanted, e.g. striatal porcine fetal ventral mesencephalic (FVM) xenotransplants for Parkinson's disease. This has no major ethical concerns and a large amount of tissue is available, however life long immunosupression and risk of rejection are the major limitations. All of these stem cells preferentially generate differentiated cells of the same lineage as their tissue of origin.



Isolation of Stem Cells

The standard method of isolating[11,12] neural stem cells in vitro is to dissect out a region of the fetal or adult brain that has been demonstrated to contain dividing cells in vivo, for example the subventricular zone (SVZ) or the hippocampus. The tissue is disaggregated and then the dissociated cells are exposed to a high concentration of mitogens such as fibroblast growth factor-2 (FGF-2) or epidermal growth factor (EGF) either a defined or supplemented medium as a matrix and as a substrate for binding. After some proliferation, the cells are either induced to differentiate by withdrawing the mitogens, or by exposing the cells to another factor that induces some of the cells to develop into different lineages.

Storage of Stem Cells

Autologous, and in some cases allogeneic, hemopoietic stem cells (HSC) are stored for varying periods of time

prior to infusion. For periods of greater than 48 h, storage requires cryopreservation. It is essential to optimize cell storage and ensure the quality of the product for subsequent reinfusion. Samples should be preserved at about -186^{0}C in liquid nitrogen for possible future use. The storage cost is expected to be very high and the demand is also extensive in India. The policy to store the umbilical cord / blood and whether it should be for general public or only for the ones with high-risk pregnancies, is yet to be defined.

A number of variables may have impact on the quality of HSC products for transplantation. These include:

- Contamination with mature blood cells; manipulations performed prior to storage, such as red cell depletion or separation of the buffy coat or mononuclear cell fractions
- The cell concentration, temperature and length of storage for products stored in liquid state prior to infusion or cryopreservation
- Cryopreservation variables - type of cryoprotectant used, rate of cooling and final storage temperature.

The quality of cryopreserved HSC is dependant on:

- The cell concentration
- Temperature
- Interval between collection and cryopreservation
- Presence of mature blood cells
- Manipulations prior to storage and cryopreservation

Bone marrow, in particular, contains a range of mature blood cells and other noncellular material. These are not optimally preserved using techniques that result in good stem cell recovery.

The presence of mature blood cells has three effects:

- Granulocytes and platelets may clump and interfere with stem cell processing.
- Red cells may lyse upon thawing and infusion, leading to renal failure.
- Large cell numbers may require freezing in large volumes, leading to volume overload and cryoprotectant-related toxicity upon infusion.

Rate of Freezing [13-15]

The main principle underlying successful cell cryopreservation is the prevention of ice crystal formation during cooling. This is a primary cause of cell damage. If cells are cooled too quickly, intracellular ice crystals form, resulting in mechanical disruption of cells and their destruction. At slow rates of cooling, ice crystals form in the extracellular space, causing increased osmolality as free water is taken up. This causes cellular dehydration. Glycerol and DMSO prevent dehydration by inhibiting the increased concentration of sodium that can occur during ice formation and by decreasing the amount of water absorbed into ice crystals at any given temperature. They are referred to as colligative cryoprotectants. Controlled-rate freezing protocols aim to achieve a rate of

cooling that minimizes intracellular ice crystal formation and protects against cellular dehydration. The optimal rate of cooling is influenced by the type of cryoprotectant and the cells being frozen. A sensible recommendation is that for HSC frozen in 10% DMSO and a minimum of 10% plasma, the optimal freezing rate should be 1-2°C/min from 0°C to −40 or −80°C.

Cryoprotectants:[16,17]

Dimethylsulfoxide (DMSO)

DMSO is a colligative agent that diffuses rapidly through the cell membrane. It has a half-life of 20 h and is metabolized to DMSO 2, which is excreted through the kidneys, whilst a small proportion of DMSO is reduced to dimethylsulfide (DMS) and excreted through the lungs, accounting for the characteristic smell. A number of studies have determined that the best concentration for DMSO or glycerol for cryopreservation of HSC is 10%, although concentrations of 5% have been used successfully.

Hydroxyethyl starch (HES)

HES is a polymer containing chains of different molecular weights. It does not freely penetrate the cell and may work by forming a viscous shell over the cell surface, inhibiting the movement of water and preventing progressive cellular dehydration. HES is generally combined with cryoprotectants such as DMSO.

Protein

Plasma proteins have cryoprotectant effects, and their addition to cryoprotectant solutions improves HSC survival. Lymphocytes can be preserved in serum alone. Most cryopreservation solutions incorporate either autologous plasma or human albumin solutions. Albumin avoids the marrow fat, cellular debris and anticoagulant contained in plasma derived from marrow collections. Autologous plasma collected at the same time as stem cells on cell separators is a cleaner product than plasma derived from marrow collections.

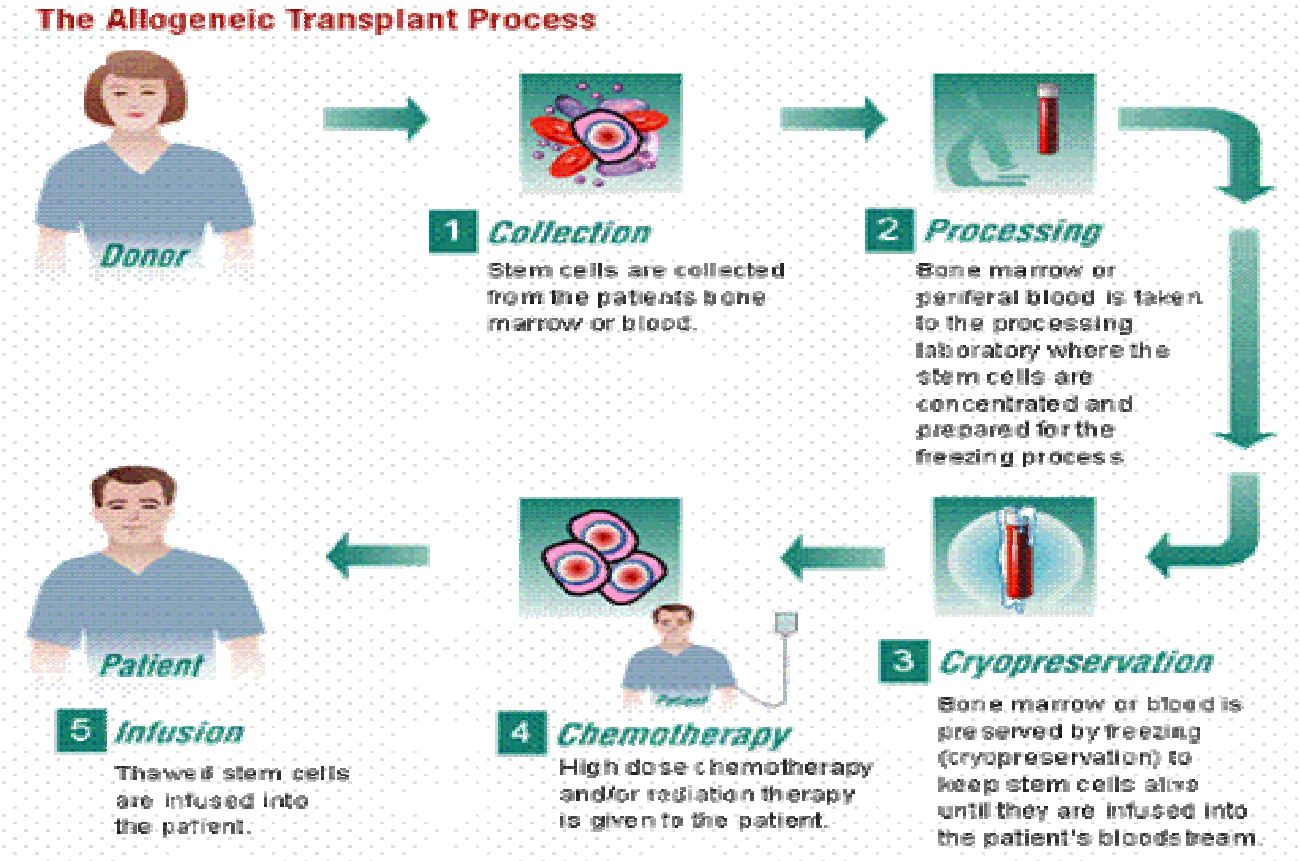
Sugars

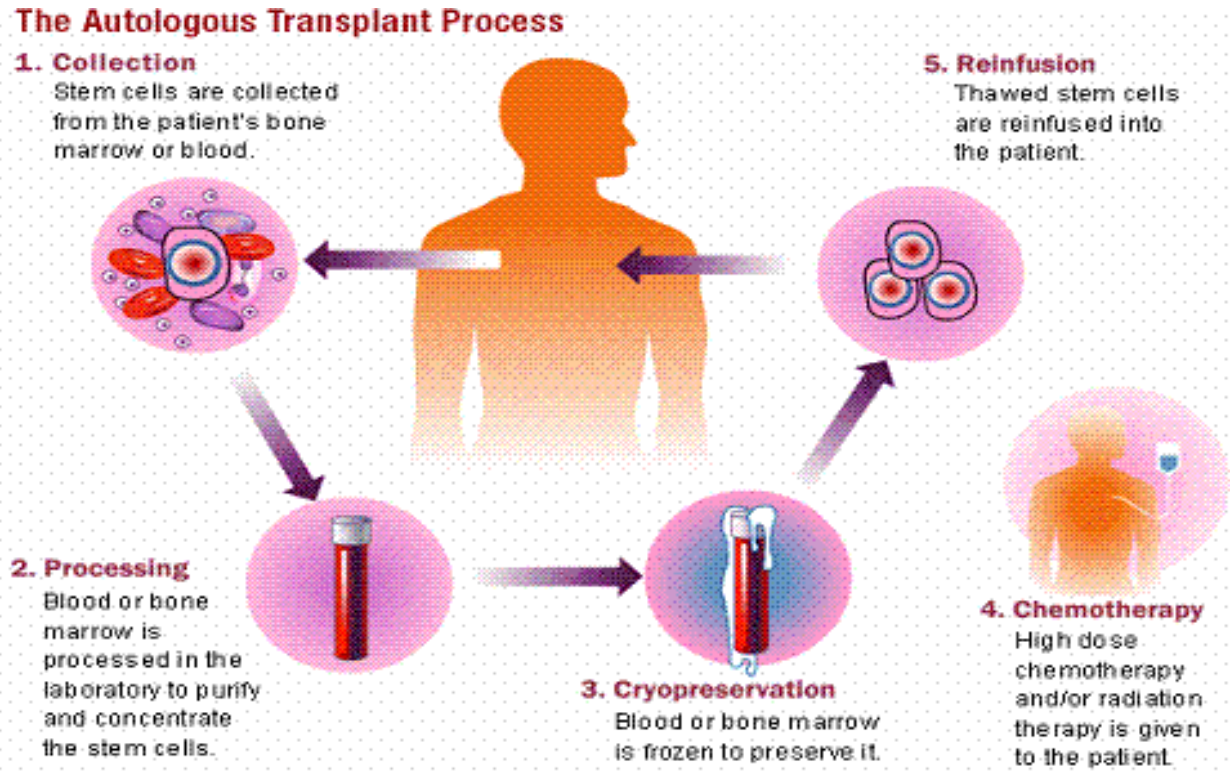
Many sugars function as cryoprotectants. In one report, 50% CFU-S survival was found when murine bone marrow cells were cryopreserved in 0.35M sucrose alone. Other sugars such as glucose, manitol and sorbitol at concentrations >0.1M also have cryoprotectant properties and may serve to stabilize the cell membrane during freezing or dehydration.

Cryoprotectant Toxicity

DMSO has a number of toxic effects; but fortunately, severe reactions are unusual. Rowley described the following types of reactions:

- Anaphylaxis
- Hypotension resulting from histamine-induced vasodilatation
- Skin flushing, dyspnea, abdominal cramps, nausea and diarrhea - also attributed to histamine release
- Cardiovascular effects: increased blood pressure, decreased heart rate, cardiac arrest, heart block
- Headache, reversible encephalopathy


The Allogeneic Transplant Process
Donor
1 Collection
Stem cells are collected from the patients bone marrow or blood.
2 Processing
Bone marrow or periferal blood is taken to the processing laboratory where the stem cells are concentrated and prepared for the freezing process.
3 Cryopreservation
Bone marrow or blood is preserved by freezing (cryopreservation) to keep stem cells alive until they are infused into the patient's bloodstream.
4 Chemotherapy
High dose chemotherapy and/or radiation therapy is given to the patient.
5 Infusion
Thawed stem cells are infused into the patient.
Patient


The Autologous Transplant Process
1. Collection
Stem cells are collected from the patient's bone marrow or blood.
5. Reinfusion
Thawed stem cells are reinfused into the patient.
2. Processing
Blood or bone marrow is processed in the laboratory to purify and concentrate the stem cells.
3. Cryopreservation
Blood or bone marrow is frozen to preserve it.
4. Chemotherapy
High dose chemotherapy and/or radiation therapy is given to the patient.

other main side effects result from hemolysis of cryopreserved red cells, causing fever; chills; hemoglobinuria; and in severe cases, renal impairment.

Assessment of the quality of stored HSC [18]
Product quality may be assessed as follows:

- Cell counts
- CD34 measurement
- CFU-GM colonies
- Engraftment

Culture of Stem Cells

MSCs are usually grown as a monolayer culture in medium typically containing 10% fetal calf serum at 37°C in a humid environment containing 5% CO_2. Standard usage of differentiation medium consists of dexamethasone, ascorbic acid-2-phosphate and beta-glycerophosphate. Mineralized deposits can appear after a week, but the treatment is often maintained for up to three weeks in order to maximize the number and size of mineralizing nodules. Additional calcium is also used to increase *in vitro* mineralization. Early signs of osteogenesis include an increase in bone-specific alkaline phosphatase activity, which can be measured enzymatically. At the end of the treatment, cells can be fixed and stained with either Alizarin-Red S solution C or silver nitrate which highlights the calcium phosphate deposits. Quantitative measurements of mineral deposition can then be obtained by colorimetry. Furthermore, osteogenic differentiation is accompanied by the expression of genes such as osterix, cbfa1, osteopontin, osteocalcin, bone sialoprotein, which can be monitored at the RNA and protein level.

Transplantation of Stem Cells

Stem cell transplantation [19,20] must be done by injecting through an intravenous tube or injected into fluid surrounding the injured part to be effective.

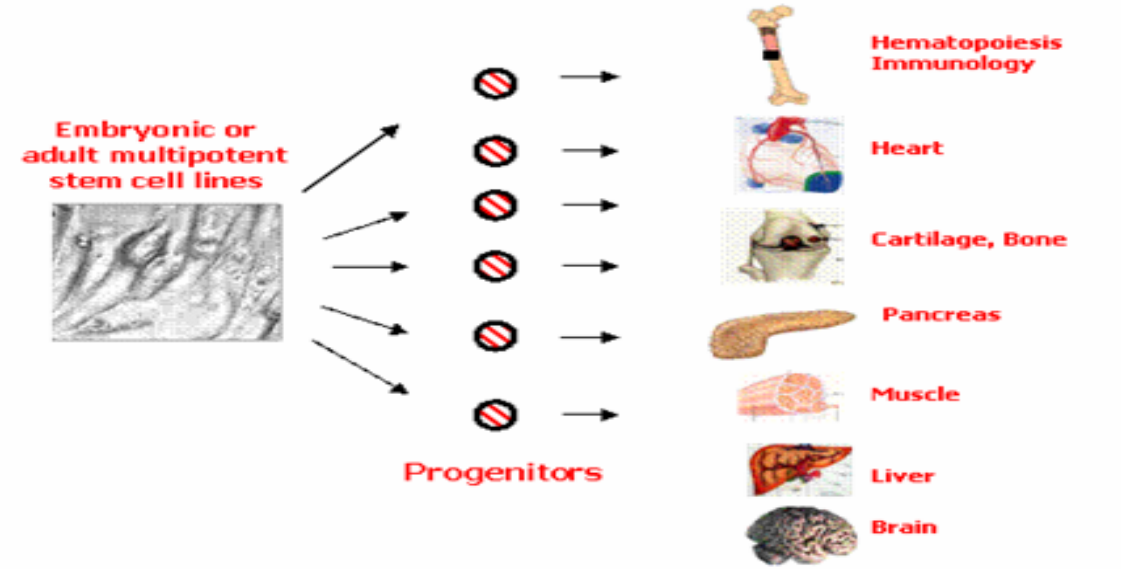
Therapeutic Uses

Umbilical cord blood stem cells have already been effectively used in the treatment of sickle cell, leukemia, non-Hodgkin's lymphoma and other forms of cancer, life threatening anemias, and autoimmune diseases. Stem cells may hold the key to replacing cells lost in many devastating diseases like Parkinson's disease, Spinal cord injury, multiple sclerosis, Alzheimer's disease, diabetes, chronic heart disease, end-stage kidney disease, liver failure and cancer. Lately stem cell use has been tried in the treatment of burns, infertility, lupus and deafness.

- **Leukemia and Lymphoma** [21,22]

Among the first clinical uses of HSCs were the treatment of leukemia and lymphoma, including Hodgkin's disease, multiple myeloma and non-Hodgkin's lymphoma. In these applications, the patient's own cancerous hematopoietic cells were destroyed via radiation

or chemotherapy, then replaced with a bone marrow transplant, or, as is done now, with a transplant of HSCs collected from the peripheral circulation of a matched donor.



- **Inherited Blood Disorders**

Another use of allogeneic bone marrow transplants is in the treatment of hereditary blood disorders, such as different types of inherited anemia and inborn errors of metabolism.
The blood disorders include aplastic anemia, beta-thalassemia, Black fan-Diamond syndrome, globoid cell leukodystrophy, sickle-cell anemia, severe combined immunodeficiency, X-linked lymphoproliferative syndrome and Wiskott-Aldrich syndrome. Inborn errors of metabolism that are treated with bone marrow transplants include: Hunter's syndrome, Hurler's syndrome, Lesch Nyhan syndrome and osteoporosis.

- **Hematopoietic Stem Cell Rescue in cancer Chemotherapy**

Chemotherapy aimed at rapidly dividing cancer cells inevitably hits other target-rapidly dividing hematopoietic cells. Doctors may give cancer patients an autologous stem cell transplant to replace the cells destroyed by chemotherapy.

- **Hematopoietic Stem Cell Therapy for Autoimmune Diseases**

The immune-mediated injury in autoimmune diseases can be organ-specific, such as type I diabetes that is the consequence of the destruction of the pancreatic beta islet cells. These autoimmune diseases are amenable to treatments involving the repair or replacement of damaged or destroyed cells or tissue. In contrast, non-organ-specific autoimmune diseases, such as lupus, are characterized by widespread injury due to immune reactions against many different organs and tissues. The objective of hematopoietic stem cell therapy for lupus is to destroy the mature, long-lived and auto reactive immune cells and to generate a new, properly functioning immune system.

- **Stem Cells and Diabetes**

Diabetes researchers have been searching for ways to replace the insulin-producing cells of the pancreas that are destroyed by a patient's own immune system. Recently, hope for a permanent cure of diabetes has appeared, namely, the transplantation of islets isolated from donor pancreas into the livers of diabetic patients. Some promising results have already been obtained with embryonic stem cells (ES cells) of both rodent and human origin. However, the potential use of ES cells for the treatment of diseases in humans is beclouded in controversy because of the ethical issues. In theory, embryonic stem cells could be cultivated and coaxed into developing into the insulin-producing islet cells of the pancreas.

- **Rebuilding the nervous system with stem cells**

The past decade has seen impressive advances in the prevention and treatment of cerebrovascular disease. Several new therapies are under investigation to address the long-term disability of stroke survivors. Stem cell therapy offers exciting potential for ambitious cellular replacement to treat diseases such as Parkinson's disease, Alzheimer's disease or even replacement of the cell death that follows thromboembolic stroke. Long-term safety and efficacy results should enhance our understanding of cell implantation therapy for the treatment of stroke.

- **Spinal Cord Disorders**

Clinicians and scientists in the field of spinal cord injury research and medicine are poised to begin translating promising new experimental findings into treatments for people. Advances in stem cell research have led to several transplantation strategies that promote axonal regrowth and partial functional recovery in spinal cord injury.

- **Repair a Damaged Heart**

For those suffering from common, but deadly, heart diseases, stem cell biology represents a new medical frontier. Researchers are working toward using stem cells to replace damaged heart cells and literally restore cardiac function. Recent interest has focused on myocardial regeneration with stem-cell transplantation as a possible treatment option to reverse the deleterious hemodynamic and neurohormonal effects that occur after myocardial infarction and can lead to congestive heart failure.

- **Orthopedics**

It is now possible to repair articular cartilage using the patient's own articular chondrocytes retrieved during arthroscopy and expanded in vitro.

- **Pulmonary medicine**

Cystic fibrosis, idiopathic pulmonary fibrosis, lung transplantation are the recent areas of pursuit.

- **Ophthalmology**

Stem cells hold promise to retinal degeneration, glaucoma and corneal disorders.

- **Parkinson's disease**[23,24]

Human precursor cells grafted into the striatum can replace degenerated dopamine-producing neurons in the nigrostriatal pathway and promote limited functional recovery.

- **Alzheimer's disease**

Stem cell therapy has also been suggested as a possible strategy for replacing damaged circuitry and restoring learning and memory and abilities in Alzheimer's disease.

- **Diseases of Bone and Cartilage**

Stem cells therapy holds promise for genetic disorders such as osteogenesis imperfecta and chondrodysplasias, as well as for conditions resulting from physical damage such as osteoarthritis and bone fractures.

- **Multiple Sclerosis**
- **Corneal transplantation**
- **Cartilage Repair**

Future Prospects

The clinical scope of the use of stem cell therapy could be endless. It may involve [25,26]

- Patients With Liver Cirrhosis Due To Biliary Atresia
- Severe Forms Of Choledochal Cysts
- Sclerosing Cholangitis
- Spina Bifida
- Hirschsprung's disease
- Renal Dysplasia (congenital dysplastic kidneys, bilateral multicystic kidney disease, polycystic disease of the kidney, severe hydronephrosis with renal insufficiency)
- Residual tumors
- Pancreatic insufficiency due to diabetes following surgical resection (nesidioblastosis, tumors)
- Neurogenic bladder
- Bowel and the postoperative neurological deficits after surgery for anorectal malformations with sacral agenesis.
- Human immunodeficiency virus type – I (HIV) infections.

Disadvantages

Any stem cell therapy will have to clear the following two hurdles:

Immune Rejection

Patients receiving a graft of embryonic stem cells or adult stem cells sourced from unrelated donors would probably be treated in much the same way that organ transplant recipients are treated. The grafts would be matched to the individual patient and anti-rejection drugs would be used.

Long Term Sequelae and Neoplastic Potential

Any stem cell, adult or embryonic, has the ammunition it needs to give rise to cancer: an explosive ability to grow and to change into other types of cells. The stem cell lines injected into patients have to be carefully tested first in animals to see if they give rise to cancer. Lymphoproliferative disease may occur during the first year, leukemia and myelodysplastic syndromes develop after several years, whereas solid tumors occur even later as long term sequelae.

Side effects of bone marrow and stem cell transplants

- Risk of infection

- Drop in red blood cells (anemia)
- Risk of bleeding
- Sickness and diarrhoea
- Sore mouth
- Difficulty eating and drinking
- Feeling tired and run down

Controversy Surrounding Stem Cell Research

There exists a widespread controversy[27-29] over stem cell research that emanates from the techniques used in the creation and usage of stem cells. Starting a stem cell line requires the destruction of a human embryo and/or therapeutic cloning. Opponents of the research argue that embryonic stem cell technologies are a slippery slope to reproductive cloning and can fundamentally devalue human life. Those in the pro-life movement argue that a human embryo is a human life and is therefore entitled to protection. Contrarily, supporters of embryonic stem cell research argue that such research should be pursued because the resultant treatments could have significant medical potential. It is also noted that excess embryos created for in vitro fertilisation could be donated with consent and used for the research.

Conclusions

Stem cells seem to be very promising in future therapeutic regimens for various life-threatening and diseases that are not curable with the currently available drugs. Though there is advanced research being taking place at a very faster pace in this field, the various ethical concerns and adverse effects should also be addressed and the best for the mankind has to be decided.

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