Stem cells: The revolution in current medicine
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Biological science is being bombarded with problems of HIV, cancers, thousands of genetic disorders, obesity, diabetes, microbial infections, biological warfare, SARS and degenerative organ diseases of the lung, liver, kidney and heart. A big question today is the promise of stem cells. Will stem cells one day be good enough to save the sinking Noah’s Ark of human health? This review attempts to give an overview of stem cells and the scientific factors revolving around it.

Keywords: adult stem cells (ASCs), embryonic stem cells (ESCs), human embryonic stem cells (hESCs), hemopoietic stem cells (HESCs), stem cell markers, pluripotency, plasticity

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Introduction

In the 1900s, biological sciences took a big leap in the field of molecular biology, with the discovery of hereditary material DNA, the blue print of life. With the most prestigious project of biology completed today i.e., the ‘Human Genome Project’, the field of therapy still has numerous unsolved mysteries ranging from treatment of common cold to HIV. In this situation today, stem cells, our own cells from human body give us a chance of hope for a healthier future. The issue of stem cell research burst on the scientific scene in 1998 when researchers reported the isolation of embryonic stem and embryonic germ cells, which offer great promise for new ways of treating diseases.

Stem cells are unspecialized or undifferentiated cells that have the unique ability to give rise to many different cell types such as skin, liver, kidney, heart, neuron or other organ cells. They also possess the property of self-duplicating for indefinite periods of times and are thus present as adult stem cells in most of the organs of the body including blood and bone marrow. Stem cells are of two types, the adult stem cells as mentioned above and embryonic stem cells, which originate from one of the earliest stages of development of the embryo, the blastocyst stage. More specifically, the embryonic stem cells (ESC’s) are derived from the inner cell mass of the blastocyst stage (Fig. 1, Table 1) i.e., before the blastocyst implants itself in the uterine wall. Most importantly, the ESC’s are pluripotent. This means that they are capable of self-renewal and differentiating into almost any cell type in the body including the cells of all the three germ layers. In contrast, the adult stem cells (ASC’s) are unspecialized or undifferentiated cells that are found in differentiated tissues. Adult stem cells usually divide to generate progenitor or precursor cells which then differentiate to develop into mature cell types that have characteristic shapes and specialized functions.

Embryonic stem cells are derived from embryos created through in vitro fertilization (IVF). IVF technology has made it possible to carry out fertilization in vitro and grow embryos in the laboratory. Traditionally, this technology has allowed for many otherwise infertile couples to have children. During this process, the embryos created offer a potential source for human embryonic stem cells. However, their use has raised ethical and policy issues, due to which their utilization is restricted.

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Table 1 — Differentiation of stem cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cell types developed</th>
<th>Differentiation conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematopoietic stem cells</td>
<td>Platelet, RBC, WBC</td>
<td>Interleukin-3, erythropoietin, thrombopoietin</td>
<td>Morrison et al 7</td>
</tr>
<tr>
<td>Mesenchymal stem cells</td>
<td>Adipocyte, chondrocyte</td>
<td>Dexamethasone, vitamin D3</td>
<td>Prockop 8</td>
</tr>
<tr>
<td>Mesenchymal stem cells</td>
<td>Skeletal muscle</td>
<td>5-azacytidine, amphotericin-B</td>
<td>Wakitani et al 9</td>
</tr>
<tr>
<td>Neural stem cells</td>
<td>Astrocyte, neuron, oligodendrocyte</td>
<td>Basic fibroblast growth factor, epidermal growth factor</td>
<td>Johansson et al 10</td>
</tr>
<tr>
<td>Embryonic stem cells</td>
<td>Adipocyte</td>
<td>Retinoic acid, T3, insulin leukemia inhibitory factor</td>
<td>Dani et al 11</td>
</tr>
<tr>
<td>Embryonic stem cells</td>
<td>Oligodendrocyte</td>
<td>Foetal calf serum (10%) , β-mercaptoethanol</td>
<td>Fairchild et al 12</td>
</tr>
<tr>
<td>Embryonic stem cells</td>
<td>Chondrocyte</td>
<td>BMP-2, BMP-4</td>
<td>Kramer et al 13</td>
</tr>
<tr>
<td>Embryonic stem cells</td>
<td>Macrophage</td>
<td>II-3, Macrophage colony stimulating factor</td>
<td>Leischke &amp; Dunn 14</td>
</tr>
<tr>
<td>Skin stem cells</td>
<td>Macrophage</td>
<td>Ectoderm skin therapy</td>
<td>Yamane et al 15</td>
</tr>
<tr>
<td>Nestin +ve islet derived progenitor cell</td>
<td>Pancreatic cell</td>
<td>NIPS isolated from pancreatic islets from long periods</td>
<td>Zulewaski et al 16</td>
</tr>
</tbody>
</table>

Fig. 1 — Origin of stem cells

Stem cells have drawn attention from 1981, when Martin Evans and Kaufman derived mouse embryonic stem cells from the inner cell mass of blastocysts 21 (Fig. 2). Table 2 gives the chronological sequence of development in the field of stem cell research. Seventeen years later in 1998, John Gearhart was able to isolate human embryonic germ cells from the gonadal ridge of 5-10-week-old fetus 29. These cells showed high proliferative capacity and multiple cell lineages 24-26,30,31. The embryonic stem cells (undifferentiated) vary in respect to their growth characteristics in vitro 13.
The fact that stem cells have the potential of developing into specific cell types and can proliferate indefinitely, makes them potentially useful in applications such as developing organs and tissues for transplantation, cell therapies for degenerative diseases, gene therapy and toxicology testing for new drugs.

**Stem Cell Markers**

Under a microscope, stem cells are similar in structure and morphology to any other cell in the tissue where they are found. So, how the researchers identify stem cells from the plethora of normal cells, especially when they occur in small numbers? answer—using the stem cell markers.

It is of common scientific knowledge that all cells in the body carry receptors on their surface. But, stem cells, depending on the site of occurrence have certain unique combinations of receptors that make them distinguishable from the normal cells. Signaling molecules are compounds, which differentially bind to these receptors. This simple principle can be used for detecting stem cells. For easier and quicker identification, these signaling molecules can be attached to fluorescent tags.

### Detecting Specific Populations of Stem Cells

With a wide range of fluorescent tags in hand, it is now very easy and convenient to identify stem cells based on receptor and fluorescent patterns. Two commonly used methods for identifying stem cells are Fluorescence Activated Cell Sorter (FACS) and Visual Assessment. FACS is a device commonly used for the separation of stem cells from the normal cells. Stem cells with the appropriate receptors specific for a tissue will bind to signaling molecules containing fluorescent tags and attain a negative charge. The normal cells remain positively charged since the fluorescent tags do not bind them. Stem cells can now be easily separated from the rest of the cell population and pooled based on the charge differences. The second method is used to assess how stem cells appear in tissues. A thin slice of tissue is treated with markers tagged with fluorescent tags and observed under microscope for emission of light of high wavelength (Fig. 3). Several other genetic and molecular biology techniques can also be used for the detection of stem cells. Markers used to identify ASCs in various tissues and organs are given in Table 3 and the commonly used markers to identify pluripotent stem cells are given in Table 4.
Prospects of Stem Cell Research in Medicine

(i) Stem Cells in Treatment and Gene Therapy

Stem cell therapy is certainly a promising area for research. Stem cells have the ability to give rise to many specialized cells in an organism. Certain types of stem cells are already used to restore blood forming and immune system function after high-dose chemotherapy for some types of cancer, and several other restorative uses have been demonstrated. The broadest potential application is the generation of cells and tissues that could be used to repair or replace damaged organs. If scientists can learn how to control stem cell conversion into new, functionally mature cells, doctors might be able to cure many diseases for which therapy is currently inadequate.

Gene therapy is an exciting option for treatment of several genetic disorders like cancer and inborn

<table>
<thead>
<tr>
<th>Tissue/organ</th>
<th>Cell type</th>
<th>Marker</th>
<th>Significance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood vessel</td>
<td>Smooth muscles</td>
<td>Cell-specific myosin heavy chain, cadherin</td>
<td>Identifies smooth muscle cells in blood vessel walls</td>
<td>Jackson et al</td>
</tr>
<tr>
<td>Bone</td>
<td>Osteoblasts</td>
<td>Hydroxyapatite</td>
<td>Markers in bone formation</td>
<td>Herzenberg &amp; De Rosa</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Bone marrow fibroblasts</td>
<td>Muc-18 or CD 146</td>
<td>Importance in hematopoiesis</td>
<td>Shamblott et al</td>
</tr>
<tr>
<td>Blood</td>
<td>WBC</td>
<td>CD 4 and CD 8</td>
<td>Markers specific for mature T-lymphocytes</td>
<td>Jackson et al</td>
</tr>
<tr>
<td>Cartilage</td>
<td>Chondrocytes</td>
<td>Sulphated proteoglycan</td>
<td>Found in connective tissue</td>
<td>Shamblott et al</td>
</tr>
<tr>
<td>Fat</td>
<td>Adipocytes</td>
<td>Fatty acid transporter</td>
<td>Transport molecule specifically located in adipocyte</td>
<td>Herzenberg &amp; De Rosa</td>
</tr>
<tr>
<td>General</td>
<td>Male cells</td>
<td>Y-chromosome</td>
<td>For detecting donor cells in female transplant recipients</td>
<td>Herzenberg &amp; De Rosa</td>
</tr>
<tr>
<td>Liver</td>
<td>Hepatocytes</td>
<td>Albumen</td>
<td>Indicates functioning of fully differentiated hepatocyte</td>
<td>Alison &amp; Ponlson</td>
</tr>
<tr>
<td>Skin</td>
<td>Epidermal cells</td>
<td>Keratin/pigments</td>
<td>For various skin damages due to burns or accidents</td>
<td>Yamane et al</td>
</tr>
<tr>
<td>Nervous system</td>
<td>Neurons</td>
<td>Neural tubulin</td>
<td>Identifies differentiated neuron</td>
<td>Woodbury et al</td>
</tr>
</tbody>
</table>

Table 4 — Commonly used (ESCs) markers to identify pluripotent stem cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Markers</th>
<th>Significance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic stem and Embryonal carcinoma</td>
<td>Oct-4, Germ cell nuclear factor</td>
<td>Essential for the establishment and maintenance of PSC’s</td>
<td>Bongso et al</td>
</tr>
<tr>
<td>Ectodermal, neural and pancreatic progenitors</td>
<td>Nestin, Vimentin</td>
<td>Found during formation of neuro ectoderm</td>
<td>Herzenberg &amp; De Rosa</td>
</tr>
<tr>
<td>Endoderm</td>
<td>α-fetoprotein, GATA-4</td>
<td>Endoderm differentiation</td>
<td>Shamblott et al</td>
</tr>
<tr>
<td>Mesoderm</td>
<td>Bone morphogenetic protein-4, Brachyury</td>
<td>Formation and differentiation of mesoderm</td>
<td>Itskovitz-Eldor et al</td>
</tr>
</tbody>
</table>
errors of metabolism. It uses genetic engineering, which is the introduction or elimination of specific genes by using molecular biology techniques to physically manipulate genetic material that may result in improved genetic constitution by adding new functions or regulate genetic activity. Gene therapy dates back to the year 1989, but its link with stem cells is fairly recent. Researchers have been using viral vectors to introduce transgenes directly into the human genome (Fig. 4). Until now about 40% of more than 450 gene therapy clinical trials conducted in the United States have been cell based. But of these about 30% have used human stem cells as the means for delivering transgenes into patients. Stem cells are basically being used to determine where the stem cells ended up and whether they were indeed producing the desired gene product and if so, in what quantities and for what length of time. The reason for using stem cells in cell based gene therapies is that they are a self-renewing population of cells and thus may reduce or eliminate the need for repeated administrations of gene therapy. Today, gene therapy has been limited to treatment of single gene disorders.

(ii) Diabetes and Stem Cells

Diabetes is a group of diseases characterized by abnormally high levels of glucose in blood stream. This excess glucose may lead to complications like blindness, kidney failure, neuropathy, etc. Type 1 diabetes or juvenile diabetes develops when the body’s immune system recognizes its islet cells as foreign and destroys them. Type 2 diabetes or adult diabetes occurs when the body cannot use insulin effectively. Both these processes build up glucose in the blood. Each year, diabetes affects more people and cause more deaths than AIDS and breast cancer combined. There is currently no cure for diabetes. Whole organ-pancreas transplants are 83% successful in-patients. However, there is very less availability of such transplants and immune rejection is also common in such cases. Transplantation of islet cells of pancreas also does not offer good results due to immune rejection. Several approaches are being used for isolating and cultivating stem cells or islet precursor cells from fetal and pancreas tissue. Periodically, stem cells may be used to develop insulin producing islet cells of the pancreas. The cells

Fig. 4 — Strategies for delivering therapeutic transgenes into patients
can be engineered to avoid immune rejections. Lamelsky et al. induced mouse embryonic cells to differentiate into insulin secreting cells; the results are encouraging in murine models.

(iii) Stem Cells in Neurobiology

Genetically engineered neural stem cells can be used in the regeneration and treatment of neural diseases. Thus, they may be used in the treatment of mucopolysaccharide and lysosomal storage diseases. Recent findings in stem cell research indicate the presence of stem cells in the hippocampus—a region in the brain, which is important in memory. Until then, it was believed that neural cells lacked the ability to regenerate. But these new findings suggest that neural stem cells have the ability to generate some, if not all types of brain cells like neurons that transmit signals and the cells that aid the nervous system, like astrocytes and oligodendrocytes. A curious observation in regeneration of neural cells is that neural stem cells in foetal and adult brain resemble the undifferentiated cells in a developing embryo that give rise to nervous tissue. This has accelerated the study of regenerating neural stem cells to unravel secrets of the most mysterious organ of the human body—the brain (Fig. 5). Pursuits in neural stem cell research mainly focus on discovering therapy using two fundamental strategies.

To grow differentiated cells in laboratory and transplant them into a diseased individual requires induction of stem cells using specific signals inside the body. After transplantation, they would differentiate into specific cell types or treat the cells in culture so that they would differentiate inside the body. The second strategy was to discover the hormones, growth factors and signaling molecules that would induce the patient’s own stem cells endogenously to migrate to the site of injury and repair and regenerate tissue. These strategies, in future, hold the promise of treating degenerative disorders of the brain like Parkinson’s disease.

Recent reports by researchers at John Hopkins University, USA, show that cells derived from ESCs can restore movement in murine model of Amyotrophic lateral sclerosis also known as Lou Gehrig’s disease. In this disease, motor neurons that control movement are progressively destroyed. Studies were conducted to see if stem cells could restore nerves and improve mobility in rats by differentiating into motor neurons to replace damaged ones that paralyzed rats, which had their motor neurons destroyed by Sindbis virus. ESCs were used that displayed the molecular markers of neural stem cells, such as protein-nestin and neuron specific enolase. Results of this study were encouraging as three months after injection, several rats were able to slowly move limbs and walk. Although these studies are preliminary and cannot be directly tested in humans, they do show some bright prospects for future human trials.

Parkinson’s disease is the most important focus of rebuilding neurons systems using stem cells. In this disease the dopamine producing neurons, deep in the brain, are gradually destroyed. These neurons connect substantia nigra in the brain to another structure called the striatum composed of caudate nucleus and the putamen. These nigro-striatal neurons release the chemical transmitter dopamine into their target neurons in the striatum. Dopamine plays a major role in controlling movement and death of such dopamine producing neurons leads to difficulty in movement, which is a characteristic of Parkinson’s disease. Levodopa, the drug used for the treatment, has several effects and has reduced effects on prolonged usage. One of the first attempts in neural cell transplantation was tried in the 80’s in Mexico when dopamine producing chromaffin cells in patients’ own adrenal glands were used to replace damaged neurons. Dramatic improvements were observed. Stem cells also offer a scope for repairing spinal cord injuries. Research in this area is still in the preclinical stage and a lot of basic work needs to be done in this area.

(iv) Stromal Cells

Stromal cells can be obtained from a small sample of patient’s bone marrow. After short proliferation
and induction they are implanted into the patient’s injured organs. Thus, patient himself can be the donor of the cells requested for transplantation at diabetes, Parkinson’s, Alzheimer’s and other diseases\textsuperscript{51}. Complex and expensive technology of obtaining ESCs, which includes human embryo cloning, can be replaced. To develop similar methods for human bone marrow stromal cells, growth factors, markers and inducers are required. Differentiation of neuroblasts from bone marrow stromal cells is shown in Fig. 5.

(v) Heart and Cardiac Muscle Repair

Myocardial infarction, i.e., heart attack and congestive heart failure are some of the most prevalent causes of death worldwide, predominantly in developed countries. Destruction of heart muscle cells—myocardiocytes due to hypertension, chronic insufficiency in heart blood supply, etc., are the major causes of these medical problems\textsuperscript{52}. Transplantation of heart, arterial stents, draining blood clots from arteries have been successful in treating the above complications\textsuperscript{53}. Stem cells offer another important solution. A number of laboratories now establish knowledge of specific growth condition in laboratory that can induce stem cells to divide into different cardiac cells like-cardiomyocytes and vascular endothelial cells. Murine models have shown encouraging results in such research and hope to be successfully extrapolated to humans in future. For example, Orlic et al used hematopoietic stem cells isolated from bone marrow through unique cellular markers to treat a murine recipient experimentally subjected to myocardial infarction. The damaged myocardium was replaced with new normal functional tissue that occupied 68% of the damaged portion; nine days after the bone marrow stem cells were transplanted\textsuperscript{51}.

Human embryonic stem cells (hESCs) proved to be a potential source of advances in cardiomyocyte regeneration\textsuperscript{53}. Itskovitz-Eldor et al demonstrated that hESCs reproducibly differentiate in culture into embryoid bodies made up of cell types from the body’s three germ layers\textsuperscript{7}. They used markers like antibodies to myosin, desmin, antinaturietic protein, etc. to identify early stage cardiac cells in embryoid bodies and carried out genetic analysis of these cells to find that transcriptional factor genes in these cells are consistent with early stage cardiomyocytes during mammalian development. These findings support the use of ESCs in treatment of heart attacks. Several studies on stem cells concentrate on determining whether the replacement of heart tissue using stem cells continues to function normally and also in finding whether the patient’s cells can be harvested and expanded for further use to minimize graft rejection. Inducing stem cells to migrate to site of injury in case of damage also provides a major issue for future. Muscle cell transplantation can thus be used as a potential therapeutic approach for treatment of patients with chronic heart failure and post-myocardial infarction, and muscle repair.

(vi) Hemopoietic Stem Cells and Immunodeficiencies

Hemopoietic stem cells (HSCs) are isolated from the blood or bone marrow that can renew itself, differentiate to a variety of specialized cells and can be released into circulating blood\textsuperscript{54}. Bone marrow transplantation for treatment of leukemia started in 1980s itself but basic research in the field of characterization of these HSCs acquired pace in 1960s itself when scientists began analyzing and identifying the components of bone marrow. They concluded that HSCs could renew themselves and give rise to different types of blood cells\textsuperscript{55,56}. The problem with HSCs is that they cannot easily proliferate for long periods, are rare and have an identity problem as they look like many other blood or bone marrow cells\textsuperscript{57}. HSCs are found in the bone marrow, peripheral blood, umbilical cord blood, fetal hematopoietic system, ESC and EGC. Umbilical cord blood and placenta are a rich source of HSCs. Presently, Reliance Life Sciences, Mumbai are studying ex vivo expansion of HSCs from umbilical cord blood. Recent reports indicate plasticity of HSCs. Lagasse and colleagues demonstrated liver repair using purified HSCs. HSCs are used since 1980s for bone marrow transplantation in leukemic individuals\textsuperscript{58}. Allogeneic bone marrow transplantation for treatment of hereditary blood disorders like aplastic anemia, β thalassemia, globoid cell leukodystrophy, severe combined immunodeficiency, etc., though have great potential but a limited scope due to significant risk of death. Joshi et al have shown that umbilical cord and peripherally harvested human HSCs show antitumour activity in the test tube against leukemic cells and breast cancer cells\textsuperscript{58}. The main focus of study of HSCs lies in mainly finding ways to safely and efficiently expanding the numbers of transplantable human HSCs in vitro or in vivo. Further advances in gene therapy techniques and understanding of cellular
plasticity could make HSCs one of the most powerful tools for healing. Future study of plasticity is hoped to aid in several other transplantations. Banked unrelated umbilical cord blood was reported to be used to reconstitute the immune system.\textsuperscript{58}

**Development of HSC Lines for Transplantation**

The ability to generate and propagate unlimited numbers of HSCs has a major impact on the safety and the availability of stem cells for transplantation. The current approach of isolating HSCs from a patient’s own peripheral blood places the patient at a risk for a flare-up of autoimmune diseases. In addition, contamination of the pure HSCs with patients mature T-cells can affect the success of treatment.

Propagation of pure cell lines in the laboratory would avoid these potential drawbacks and increase the number of stem cells available to each patient, thus shortening the risk-interval before immune reconstitution. Pure HSCs lower the incidence of graft versus host rejection, which is a fatal complication of transplantation. Ultimately, stem cell gene therapy allows for the development of novel methods for immune modulation in autoimmune diseases.

**Sources of HSCs:**

a) Bone marrow – About 1 in every 1 lakh cells in the marrow is a long-term blood forming stem cell, other cells include the stromal cells and blood progenitor cells.

b) Peripheral blood – A small number of stem and progenitor cells circulate in the blood stream; the peripherally harvested cells contain twice as many HSC’s as stem cells taken from bone marrow and engraft more quickly.

c) Umbilical cord blood – Cord blood and placenta are rich sources of HSC’s. It is also suggested that they also contain stem cells, which are multipotent.

**Stem Cells and Cancer**

Stem cells have gained a lot of interest in cancer research because of their self-renewing capacity similar to that of cancer cells. There are three aspects that the researchers have focused their interests on. Firstly, find the similarities in the mechanism that regulate the self-renewal of normal stem cells and cancer cells. Secondly, how the cancer stem cells arise from normal stem cells and lastly the possibility that the tumours contain cancer stem cells.\textsuperscript{13}

The pathways associated with cancer have been found to regulate stem cell renewal also. Three signaling pathways [Notch, Sonic hedgehog (shh) and Wnt] have been looked at and they have shown to increase the self-renewing capacity of the HSC’s in vitro.

As the pathways regulating stem cell self-renewal and tumorigenesis are the same, there is every possibility that the dysregulation of these pathways lead to tumorigenesis.\textsuperscript{59} Moreover, as stem cells persist for longer periods of time there is much greater opportunity for them to get mutated and thus lead to cancer. Extensive research is being carried out in this regard.

**Renal Cell Carcinoma**

Nonmyeloablative allogeneic stem cell transplantation can induce sustained regression of metastatic renal cell carcinoma in-patients who have had no response to conventional immunotherapy.\textsuperscript{23}

**Brain Tumours**

Combination of high-dose chemotherapy with stem cell transplants from the patients themselves shows good response in the treatment of brain tumours.\textsuperscript{49}

**(viii) Stem Cell Based Therapies in Autoimmune Diseases**

Autoimmune diseases like multiple sclerosis, systemic lupus erythematosus, juvenile rheumatoid arthritis and rheumatoid arthritis may have a possible permanent cure in future. High-dose chemotherapy followed by autologous HSCT is feasible and safe, and can result in long term improvement of disease activity in patients whose condition previously did not respond to conventional antirheumatic drugs or TNF blocking agents. Autoimmunity that arises from the cellular mistaken identity lowers the body’s immune response and may lead to life-threatening complications. Stem cells may be used to remove misguided immune cells and strategically restore the normal immune cells. All the immune and blood cells develop from the multipotent hemopoietic stem cells that originate in bone marrow. Immune tolerance ensures that cells do not attack self-proteins of the body.\textsuperscript{53} Autoimmune diseases arise when this intricate tolerance system of the body fails. The diseases (e.g. rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, etc.) result in cell and tissue destruction. Currently, autoimmune diseases are treated using anti-inflammatory drugs, immune suppressive and immune modulatory agents.\textsuperscript{57}
However, such therapies do not give a significant clinical improvement. Stem cells offer a potential to alleviate these chronic diseases. SLE is a severe autoimmune disease, which affects multiple organs. HSCs can be used for replacing harmful autoimmune cells thus minimizing the effect of SLE. Replacement therapy can also be used to decrease the recurrence of disease. Experimentally, genetically modified HSCs are giving encouraging results. However, usage of stem cells in treating autoimmune diseases like SLE, RA is only in the preliminary stages. ESC could be used in preventing graft rejection in humans.

(ix) Mesenchymal Stem Cells and Joint Repair

Stem cells can be used in the regeneration of severely damaged meniscus to prevent osteoarthritis, therefore, helping in the regeneration of diseased bone tissue. MSCs in addition to their ability to support hematopoiesis can differentiate into osteocytes, chondrocytes, adipocytes and smooth muscle cells.

(x) Adult Stem Cells for New Corneas

Researchers in the United States and Taiwan have used corneal adult stem cells to grow new corneas for patients with previously untreated eye damage. Amniotic membrane transplantation appears to be a safe and effective method of restoring a stable corneal epithelium for cases of partial limbal stem cell deficiency and can be considered as an alternative to limbal auto graft or allograft. The ocular surface is made up of two distinct types of epithelial cells, constituting the conjunctival and the corneal epithelia. These epithelia are stratified, squamous and non-keratinized. Although anatomically continuous with each other at the corneoscleral limbus, the two cell phenotypes represent quite distinct subpopulations. The stem cells for the cornea are located at the limbus. The microenvironment of the limbus is considered to be important in maintaining stemness of the stem cells.

(xi) Endothelial Progenitor Cells

Endothelial cells line surfaces of blood vessels throughout the body. During embryonic development, just after gastrulation, a kind of cell called hemangioblast which is derived from mesoderm, is presumed to be the precursor of haemopoietic and endothelial cell lineages, they can give rise to both blood cells and blood vessel cells. VEGF and FGF-2 play critical roles in the endothelial cell differentiation. Delivery of young bone marrow-derived stem cells offers a novel approach for restoring the impaired senescent cardiac angiogenic function that may underlie the increased morbidity and mortality associated with ischemic heart disease in older individuals.

(xii) Skeletal Muscle Stem Cells

At least three populations of skeletal stem cells have been identified as the satellite cells, cells in the wall of the dorsal aorta and side-population cells. Satellite cells are stimulated to divide in the event of injury and give rise to myogenic precursor cells, which differentiate into the basal lamina of mature muscle cells or myofibril. Side-population cells can also regenerate skeletal muscle and can be separated by FACS. Thus, mesodermally derived tissues may be able to generate skeletal muscle.

(xiii) Epithelial Cell Precursors in Skin and Digestive Systems

Epithelial cells, which make up 60% of the differentiated cells in the body, are responsible for covering the internal and external surfaces of the body. The epithelial cells in the skin and the digestive tract are replaced constantly by the crypt cells, which are thought to be the stem cells giving rise to structurally proliferating units. Stem cells in the bulge region of the hair follicle give rise to multiple cell types. Their progeny can migrate down to the base of the follicle where they become matrix cells, which can give rise to different cell types in the hair follicle and also may give rise to the epidermis of the skin.

(xiv) Stem Cells in Pancreas and Liver

In adult mammals, both pancreas and the liver contain multiple kinds of differentiated cells that may be repopulated by multiple types of stem cells. Stem cells in the adult pancreas are postulated to occur in the pancreatic ducts of islets. Recent studies in rodents indicate that HSC’s (mesodermal origin) demonstrate the property of plasticity and give rise to hepatocytes (endodermal origin; Fig. 6). In vivo occurrence of such phenomenon is not identified. Hepatocytes themselves may be responsible for the regenerative capacity of liver.

The potential of stem cells, as possible treatments have been in the forefront of scientific research in recent years. As ESCs have the tremendous ability to develop into specialized cells, they may be used in the treatment of certain debilitating illnesses like Parkinson’s and Huntington’s diseases. Among the somatic/adult stem cells, epithelial stem cells are being used in tissue engineering for the renewal of
Fig. 6 — Progenitor-cells of hepatocytes differentiated from bone marrow stromal cells in condition medium. Staining by methylene blue and methylene violet

skin. Stem cell therapy offers the possibility of transforming stem cells into specialized cells that can be used to replace or repair diseased, injured or worn out body tissue, enabling doctors to treat devastating and currently incurable diseases.

Where is Stem Cell Research Going on?

Several companies like Advanced Tissues Sciences, Inc. (La Jolla, CA) and Organogenesis, Inc. (Canton, MA) manufacture a commercially available skin substitute from stem cells. With the August 9th announcement by President Bush, Federal funding of stem cell research in United States has been limited to the 64 cell lines known until then. Two laboratories in India, the Reliance Life Sciences Laboratory in Mumbai and the National Centre for Biological Sciences at Bangalore have 7 stem cell lines, Bresa Gen, an Australian Company has 5, Goteburg University in Sweden 19, the Karolinska Institute, 5 and Technion Institute in Haifa, Israel 4. On the whole, institutes in five countries in the world control the 64 stem cell lines included in the NIH list. The institutes engaged in stem cell research in India are given in Table 5.

Ethical Aspects

The main ethical issues for consideration are as follows:

1. Is it acceptable to reprogramme a human embryo so that instead of producing a baby, it develops only into certain types of cells?
2. Having decided that the reproductive cloning of human beings is unacceptable, is it permissible to use nuclear transfer procedures to create cloned human embryos for the sole purpose of producing human stem cells? In either case, are there likely to be viable alternative therapeutic methods, which could avoid using embryos?
3. If the route to such alternative methods involved some limited embryo research, would such research be permissible?
4. Would it be acceptable to perform the nuclear transfer of human cells into the enucleated egg of a cow, to produce a non-viable chimera, which would be reprogrammed to produce certain human cells?
5. Would the risks involved in cell replacement therapy be considered acceptable?

Due to the ethical and moral aspects involved in using human ESCs, the research in this field is currently very limited. In light of this, some scientists use ASCs as a less controversial alternative. In the United States, the Federal Government took a major decision when President Bush announced that US Federal funding for stem cell research would be restricted to the 64 cell lines known around the world presently. This ethical issue has divided the scientific community into two schools of thought—those that believe in the magnificence and of stem cell research and thus do not believe in the ethical ambiguity, and those who are apprehensive about the prospect and thus take a moral stand on research against using ESCs. In India, wasted embryos available from IVF clinics only are permitted to be used by researchers after receiving consent from the donors. However, more recently, Indian Council of Medical Research, New Delhi has formulated guidelines for stem cell research in the country.

Ethics of Stem Cell Research

Some opponents of ESC research argue that research on stem cells obtained from adults is just as promising and renders ESC research unnecessary. Most scientists, however, dispute this claim, citing great potential in the field of adult stem cells but several drawbacks as compared with ESCs. Proponents of ESC research advocate funding for both fields.
however, necessarily takes as its starting point, the current scientific understanding that it cannot be presumed that ASCs would be universally productive in this way. Indeed, a number of researchers suggest that different diseases may require different routes for producing the relevant replacement cells. On present evidence, these would probably inevitably involve some use of embryos\textsuperscript{12,14}.

If a course of action with profound ethical difficulties were to be pursued, it is also essential to be honest about its chances of success. There is a formidable list of experimental hurdles to overcome. No one knows how successful cloned cells would be on patients, or what risk there is of cultured cells becoming cancerous, as the \textit{New Scientist} pointed out. There is a risk of raising expectations too high among those suffering from the relevant diseases.

**Assessing Human Stem Cell Safety**

With the cultivation of stem cells, their use in transplantation does not proceed as smoothly as expected. With the use of mammalian cells into other hosts, several safety issues come into picture. Donor screening is a crucial step in cultivation. For instance, in case of human ESC and ASC, the donor cells must be tested for the presence of infectious agents before establishing cell lines. Further pedigree assessment to identify any genetic disorders in family history and molecular genetic testing are also mandatory. These tests are especially required when transplantation is aspired for.

The medium, for culturing human ESCs and human EGCs, requires a feeder layer of irradiated mouse embryonic fibroblast cells. This has a risk of transferring animal viruses to humans, unintentionally. Researchers are today looking for an alternative to this feeder layer. Geron Corporation, a Biotech company in USA has demonstrated use to basement membrane matrix, which may substitute for the mouse feeder layers. Detailed characterization of human stem cell populations helps in assuring stem cell safety. A panel of orthogonal assessments like visual inspection of cells to assess their appearance, expression of unique cell surface antigen, characterization of biochemical markers, such as tissue specific enzyme activity (e.g. enzymes that produce neurotransmitters, etc.) and expression of genes in a given cell type are important in this regard. Assays establishing the function of a given human stem cell prepared to be used as a transplant are important in predicting its fate in the host. Complete characterization of stem cells to evaluate safety and efficacy in host should hence be demonstrated before any further therapeutic advancement.

**Patenting Human Cells**

With the rapid advancement in the field the race for discoveries in stem cell research, patenting of respective cell lines has also commenced. From the early days of ESC research itself patents have been granted to several pioneers in the field like James Thomson at Wisconsin, Aastrom Biosciences, etc. But
such patents protect several fundamental technologies based on stem cells and are thought to pose some threat to further research in the field. In future with further discoveries in the field, patenting could also determine the path of stem cell research.

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