

# Stem cells: Concepts and prospects

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## 1. Introduction

Regenerative therapy for organ dysfunction is a rapidly growing domain and involves application of multiple enabling technologies incorporating stem cells, genes and growth factors that can accelerate the recovery of a failing organ through cell and tissue regeneration within the organ. Several strategies are currently being evaluated for regeneration of damaged organs. These are aimed at ‘reviving’ existing malfunctioning cells, repopulating the organ by new cells from exogenous or endogenous sources, altering the extracellular matrix, or increasing blood supply by enhancing vasculogenesis. Stem cells with their unique and facile potentialities, offer building blocks for organ development and tissue repair.

Over the years, a number of preclinical and small clinical trials have shown that tissue regeneration can be induced when stem cells of various types – embryonic stem cells, stem cells from cord blood and bone marrow, and adult stem cells – are injected into injured or degenerated tissue. Several small clinical trials have reported varying degrees of functional improvement. There are also attempts to use the progenitor cells to deliver functional genes as well as to seed the progenitor cells onto implants to improve the biocompatibility of implants. The promising results from many centers involved in the treatment of end-stage diseases using stem cells highlight the wide range of possibilities in this field. Despite its very early stage, almost every major medical center across the globe is involved in at least one cell

therapy effort. Companies are viewing at multi-billion dollar markets in cell therapy in the future.

This review summarizes the current concepts in stem cell biology and important advancements and limitations with respect to their prospective use in regeneration therapies in various human diseases.

## 2. Historical perspective

Rudolf Ludwig Karl Virchow (1821–1902), the founder of cellular pathology and the one who pioneered the modern concept of cell theory (*Omnis cellula e cellula*) originally proposed the concept of ‘stemness of each cell from another cell’. His student, Julius Friedrich Cohnheim (1839–1884) studied the cells appearing in wounds and concluded that they originate from the bloodstream and, by implication, from the bone marrow. The use of human stem cells for treatment dates back to the 1950s. A team led by Nobel laureate E Donnall Thomas at the Fred Hutchinson Cancer Research Center demonstrated that bone marrow cells infused intravenously could repopulate the bone marrow and produce new blood cells in patients who had bone marrow depression following chemotherapy. The first quantitative descriptions of the self-renewing activities of transplanted mouse bone marrow cells were documented a decade later by Canadian researchers Ernest A McCulloch and James E Till. Studies by McCulloch, Till and colleagues demonstrated for the first time the clonal nature of haematopoietic development. They showed that single bone

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marrow-derived cells (now called hematopoietic stem cells) could give rise to colonies of differentiated blood cells [1,2]. In 1976, Friedenstein and coworkers isolated the multipotent mesenchymal stem cells from the bone marrow and discovered their ability to develop into a mature bone tissue *in vivo* [3]. A plethora of strategies and technologies are available now for isolation and expansion of adult stem cells (or the somatic stem cells) from various sources. The normal physiologic function of the stem cells in an adult organism seems to be maintenance and repair of their tissue of origin. The general contention is that adult tissue-derived stem cells are developmentally restricted to the tissue where they reside. Recent studies however provide evidences to suggest that under appropriate conditions, some populations of adult stem cells are endowed with the capacity to transdifferentiate into cells similar to pluripotent embryonic stem cells.

Interest in pluripotent embryonic stem cells was stimulated by the isolation of stem cells from mouse teratocarcinomas, gonadal tumours containing differentiated somatic tissues such as nerve, bone, muscle, etc., sometimes, embryoid bodies, and also undifferentiated elements composed of embryonic carcinoma (EC) cells (the key malignant pluripotent stem cell of these tumours). The recognition that EC cells are the malignant counterparts of embryonic inner mass cells (ICM) eventually resulted in the experiments of Evans and Kaufman and also Martin in 1981, who showed that it is possible to derive permanent lines of cells directly from mouse blastocysts, which closely resemble the EC cells derived from teratomas [4,5]. They termed these cells as mouse embryonic stem cells (ESCs). The potential therapeutic applications and the unique opportunity to study early mammalian development exemplified by murine experiments motivated the researchers to establish human ESC (hESC) lines in a similar manner [6,7]. According to a recent review, more than 300 cell lines have been reported worldwide [8]. The most exciting properties of the hESCs lie in their potential to differentiate *in vitro* to cell derivatives of all three embryonic germ layers. Since the initial report of the derivation of hESCs, using both spontaneous and induced *in vitro* differentiation systems, these cells have been shown to transform into virtually any type of tissue such as cardiac tissue [9], neuronal tissue [10], hematopoietic progenitors [11], keratinocytes [12], endothelial cells [13], etc.

### 3. Defining the 'stem cell'

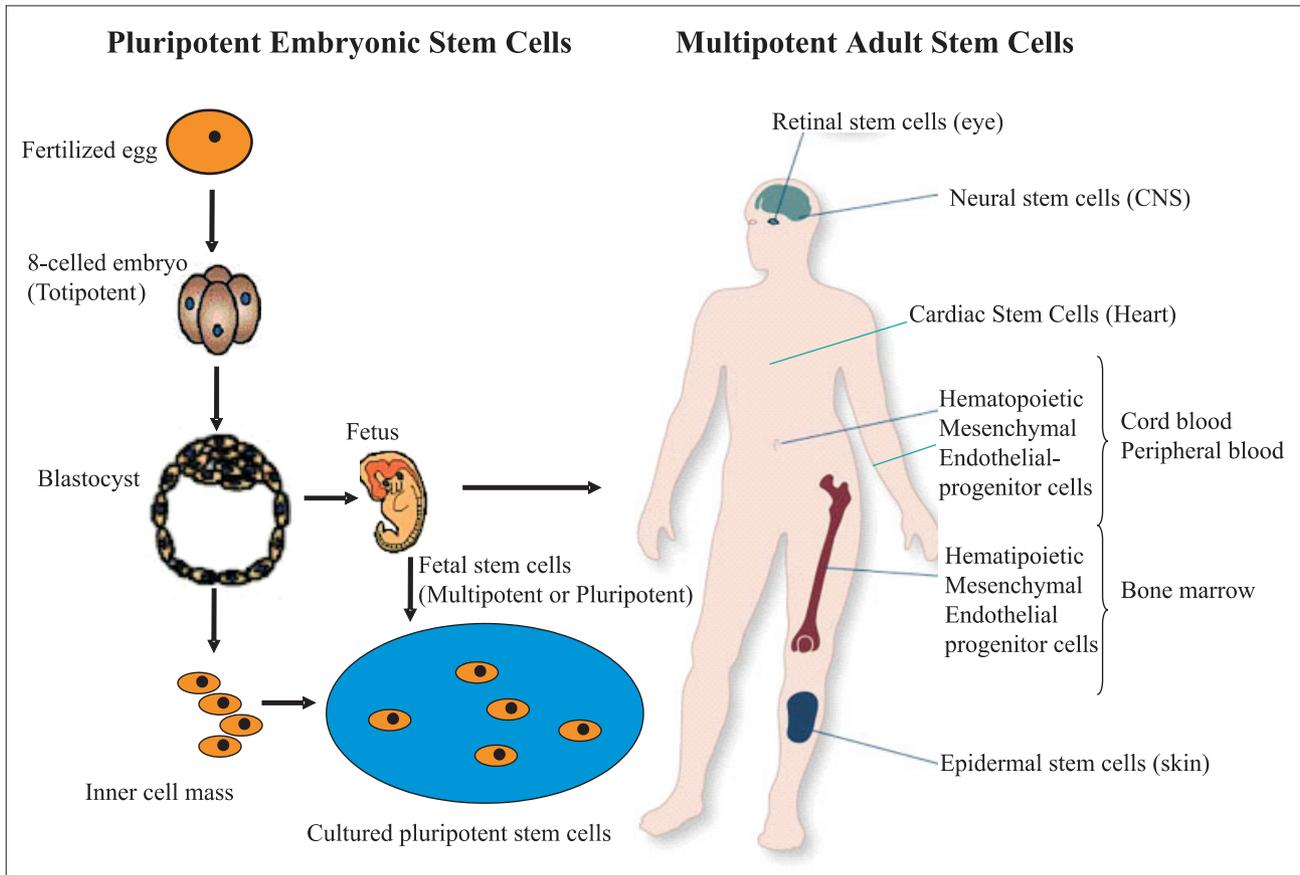
A 'true stem cell' must comply with the following stringent criteria. It must be unspecialized,

clonogenic and capable of unlimited self-renewal, a process during which a stem cell can divide symmetrically and give rise to two daughter stem cells. It is this capacity to self-renew over a prolonged period of time that ensures that stem cell populations last throughout the life of an organism. Further, it must also be able to divide asymmetrically and give rise to one daughter stem cell and the other daughter cell that in response to appropriate signals can differentiate into multiple types of differentiated cells of all three primitive embryonic germ layers (the ectoderm, mesoderm, and endoderm).

### 4. Sources of stem cells

Searches for adult stem cells have relied on information derived primarily from studies of stem cells in the bone marrow. Two important heterogeneous populations of stem cells in the bone marrow include hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). HSCs give rise to all the blood cell types including erythrocytes, monocytes, neutrophils, eosinophils, basophils. MSCs provide stromal support to the HSCs and are progenitor cells for types of cells such as osteoclasts, chondrocytes, myocytes, etc. These cells give rise to intermediate precursor- or progenitor-cell populations that partially differentiate and commit to various cell lineages. Recently various subsets of HSCs and MSCs have been isolated from bone marrow and blood. Besides the bone marrow and the peripheral blood, multipotent adult stem cells can be isolated from other tissues of the body (figure 1). For example, multipotent MSCs have been isolated from fetal liver, umbilical cord and adipose tissue [14]. A naturally rejuvenating tissue such as the skin comprises a rich source of epidermal stem cells. Neural stem cells have been isolated from specific regions of the brain, cardiac stem cells from atrial biopsies and retinal stem cells from the eye. Although, the origin of stem cells in the bone marrow is well established, the location of stem cells in other tissues remains elusive. It is hypothesized that our bodies have retained a population of reserve stem cells; perhaps set-aside during gestation and that these cells might be coerced into renewed regeneration later in life.

Early hematopoiesis occurs simultaneously in multiple organs, which includes the yolk sac and aorta-gonad-mesonephros region. These regions are critical in establishing the blood system in the embryos and lead to the eventual movement of stem cells into the fetal liver. Stem cells isolated from these fetal tissues are known as fetal stem cells. Since isolation of fetal stem cells from human fetuses is highly controversial, various other



**Figure 1.** Various stem cell types from different sources.

sources of fetal stem cells have been identified. For example, hematopoietic and mesenchymal fetal stem cells with pluripotent cell-like properties from the cord blood have been identified [15]. There is also the potential to harvest fetal stem cells from discarded placental tissues [16].

ESCs are derived from the ICM of preimplantation blastocyst stage; the ICM is separated from the trophectoderm (which would develop into the extra-embryonic tissues) using immunosurgery and mechanical dissection. These ICM cells are then plated onto a feeder layer of cells that supply both soluble factors and contact-mediated support. The ICM cells attach and over time expand to form an ESC line [6]. Initially, murine embryonic fibroblasts were used as feeder cells for the hESCs, and they still remain a good option for many research applications [6]. Newer protocols are however relying more on mechanical separation of the ICM and many new feeder layers, including human fibroblasts, human fetal tissue and non-cellular layers made up of basement membrane proteins are being used [17].

More recently, new sources of pluripotent stem cells have been discovered. Guan *et al* have successfully produced several lines of pluripotent stem cells from spermatogonial (sperm-producing)

cells that largely mimic the abilities of ESCs [18,19]. Meng *et al* have discovered a population of stem cells in the menstrual blood [20]. These cells named as the 'Endometrial Regenerative Cells' are capable of differentiating into nine tissue lineages namely, cardiomyocytic, respiratory epithelial, neurocytic, myocytic, endothelial, pancreatic, hepatic, adipocytic and osteogenic.

While ethical debate on the propriety of isolation of ESCs from human embryos continues, induced pluripotent stem cell lines have successfully been derived by inducing the expression of pluripotency related genes in the somatic cells. These cells designated as induced pluripotent stem cells (iPS) exhibit morphology of embryonic stem cells and express ES cell markers [21,22].

## 5. Types of stem cells

Depending on their regenerative potency, stem cells are classified as totipotent, pluripotent, or multipotent stem cells.

Totipotent stem cells have the potential to become any kind of cell in the body. After an egg is fertilized, it undergoes a series of divisions to become an embryo and later a fetus. The cells

that are formed during these first few divisions are totipotent, i.e., each cell can form a complete organism.

Pluripotent stem cells result after totipotent stem cells undergo the first few divisions. Pluripotent stem cells include cells from the blastocyst stage of the embryo. Given the right signals, a pluripotent stem cell could turn into any cell in an organism (except placenta), potentially growing into tissue for a heart, a kidney or bone.

Multipotent cells can be isolated from many tissues of the body and function as a repair system for damaged tissue. As compared to the totipotent and pluripotent stem cells, they possess a limited ability to differentiate into other cell types. Adult stem cells from the blood, nervous system and heart represent multipotent stem cells.

## 6. The stem cell niche

Stem cells are defined by their function in complex multidimensional environments termed as stem cell niches. The simple location of stem cells is not sufficient to define a niche. The niche must have both anatomic and functional dimensions, specifically enabling stem cells to reproduce or self-renew. Adult stem cells generally have limited function without the niche. For example, HSC, which regenerates the entire blood and immune system, circulate freely, but seem to have little function outside specific anatomic locations. It is specific cues from specific sites that allow stem cells to persist, and to change in number and fate. Importantly, it is also the niche that provides the modulation in stem-cell function needed under conditions of physiologic challenge. The ability of the niche to impose functions on stem cells makes them relevant in disease conditions. The concept of a niche as specialized microenvironment housing stem cells was first proposed by Schofield [23]. Experimental evidence was first provided in the invertebrate models, thirty years later [24,25]. In invertebrate models, it has been demonstrated that niche is composed of heterologous cell types. This has led to search for niche components in mammalian tissues and identification of the osteoblast in the bone marrow, and the endothelium in the brain, and possibly in the bone marrow [26–28].

Cells, matrix glycoproteins and the three-dimensional spaces form a stem-cell niche. The contact between these elements allows molecular interactions that are critical for regulating stem-cell function. Secreted proteins offer a paracrine measure of control, but non-protein components of the local microenvironment also affect stem-cell function. Among the matrix proteins,  $\beta$ -1 integrins in the skin and tenascin C in the nervous system

have been identified to affect stem cell number or function and participate in several stem cell niches [29–31]. Soluble mediators of cellular function in the stem cell niche have also been defined. Bone morphogenetic proteins (BMPs), wingless-related proteins (WNTs) and their antagonists, soluble notch modulators, fibroblast growth factors (FGFs) and Hedgehog (HH) contribute their inputs in a paracrine manner. They have varying capacity to induce proliferation or impair differentiation [32,33]. Metabolic products such as calcium, oxidative stress and levels of reactive oxygen species are also known to markedly affect stem-cell function [34,35]. It is expected that further understanding of the role of the stem cell niche would pave way for novel therapies to enhance and improve the regenerative capacity of stem cells.

## 7. Stem cell therapy for regeneration

### 7.1 Hematological

Allogeneic HSC transplantation or bone marrow transplantation (BMT) has now become an effectively established curative treatment of genetic and malignant hematologic disorders. Significant and sometimes even substantial improvements have in fact been achieved, albeit to different degrees for different diseases, with allogeneic transplant of HSC over the last 10–15 years. The significant impact can be deduced from data related to pediatric patients affected by severe combined immune deficiency (SCID) and severe aplastic anemia (SAA) [36,37]. In the former group, the cumulative probability of survival in patients treated by BMT from an identical sibling, estimated as roughly 60% until 1982, has risen above 95% since 1983. For patients with SAA, the increase in disease-free survival has been from 49% in the period 1970–1980 to 70% in the period 1981–1983 and to 81% over the next five years (1984–1988). There have been less significant improvements in patients with acute lymphoblastic leukemia (ALL) given an allogeneic BMT from an HLA-compatible relative. In these patients, according to the data provided by the AIEOPBMT registry, the cumulative probability of leukemia free survival was 42% in the period between 1985 and 1990 and has increased only to 50% in the period 1991–1995 [38].

Besides BMT, the two other most widely practised transplantation techniques include transplantation of circulating progenitor cells (CPCs) from peripheral blood and umbilical cord blood cells (UCBC) (table 1).

Autologous CPCs are increasingly being used following high-dose therapy for malignant disease,

**Table 1.** Status of clinical trials using different types of stem cells in human diseases.

Human diseases	Types of stem cells in use (Experimental and clinical studies)	Status of clinical trials
Hematological	Bone-marrow derived stem cells Peripheral blood stem cells Cord blood stem cells	Phase III using HLA-matched HSCs* from all three stem cell sources, Phase-II/III allogenic, haploidentical HSCs in malignant and non-malignant hematological disorders
Corneal and Retinal	Corneal stem cells Adult epithelial stem cells or Retinal pigment epithelial cells Embryonic and Adult Neural Stem Cells	Phase II/III using <i>ex vivo</i> cultured human limbal epithelial stem cells for patients with limbal stem cell deficiency Phase I/II using retinal pigment epithelial cells in patients with retinal degeneration
Cardiovascular	Hematopoietic stem cells Mesenchymal stem cells Endothelial progenitor cells Cardiac stem cells Embryonic stem cells Fetal cardiomyocytes Skeletal myoblasts	Phase I/II using autologous skeletal myoblasts, bone marrow stem cells, peripheral blood and adipose-derived stem cells in patients with myocardial ischemia, myocardial infarction, coronary artery disease, heart failure
Neurological	Mesenchymal stem cells Embryonic and Adult Neural Stem Cells Embryonic stem cells	Phase I/II using autologous bone marrow stem cells in patient with ischemic stroke, multiple sclerosis, spinal cord injury
Muscoskeletal	Mesenchymal stem cells Muscle-derived stem cells Bone marrow-derived side population cells Vascular wall stem cells	Phase I/II using autologous bone marrow stem cells in patients with critical limb ischemia, Phase I using muscle-derived stem cells in patients with Duchenne muscular dystrophy, Phase I/II and II/III using MSCs# in patients with long bone defects and articular cartilage defects
Renal	Hematopoietic stem cells Mesenchymal stem cells Endothelial progenitor cells Embryonic renal cells	Clinical trials using MSCs yet to be initiated

\*HSCs: hematopoietic stem cells; #MSCs: mesenchymal stem cells

because of the ease of collection and the markedly faster kinetics of engraftment in comparison with bone marrow [39]. In childhood autologous transplantation, CPCs have been mobilized into peripheral blood and collected on a large scale by leukapheresis after treatment with hematopoietic growth factors [40,41]. Recently, CPCs have been considered as an alternative to bone marrow for allogeneic transplantation and this procedure is also being used increasingly in adults [42]. Although there is no definitive proof from controlled clinical studies, allogeneic transplant of CPCs has some undisputed advantages in comparison with BMT; for the recipient the duration of neutropenia and of thrombocytopenia is reduced, and for the donor the trauma of harvesting marrow from the bone, with associated inevitable anesthesia, is eliminated.

The other most significant alternative to BMT (which is now used routinely) is UCBC transplantation, which was introduced by Gluckman *et al* in a 5-year-old child affected by Fanconi's anemia. The child was given an allogeneic transplant

of HSC using the cord blood of a healthy, HLA-compatible sibling [43]. Wagner *et al* has demonstrated the applicability of the procedure even in adults [44]. The cells obtained from a cord may however be quantitatively insufficient for quick engraftment of the transplant in most adult patients, a great limitation in terms of routine use of the cord cells [45]. UCBC transplants are currently performed, both from an HLA-compatible family donor and from an unrelated donor. Improvements in the methods used for cell collection, manipulation and freezing have allowed a rapid increase in the use of UCBC. Reported low incidence of acute and chronic graft versus host disease has promoted the establishment of large cord blood banks in Europe and the USA, where at present more than 12,000 cord blood units have been collected and typed for the HLA system [46]. Significant expansion of CB progenitor cells *in vitro* is also possible now with the use of a combination of cytokines and chemokines [47]. Despite the impressive effect seen *in vitro*, clinical benefit with the expanded cells however do not seem to differ

much from that obtained with the unmanipulated cells [48].

### 7.2 Corneal and retinal

Repair of degenerative diseases of the eye is a prime example of stem cell therapy in routine, effective clinical practice. Both corneal and retinal stem cell populations have been identified in the adult human eye. The corneal epithelial cells have a finite life span and are continuously renewed by proliferating stem cells in the limbus located at the junction between the cornea and the conjunctiva [49]. The corneal stem cells constitute between 0.5 and 10% of the total cell population in the epithelial tissue. Under certain conditions, however, the limbal stem cells may be partially or totally depleted resulting in varying degrees of stem cell deficiency with accompanying abnormalities in the corneal surface. Such deficiency leads to conjunctivalization of the cornea with vascularization, appearance of goblet cells and irregular and unstable epithelium. Stem cells can be delivered by limbal auto or allo grafts depending on the source of the donor tissue [50–53]. Transplantation of *ex vivo* expanded donor limbal cells is another strategy to provide the limbal tissue [54,55]. Amniotic membrane harvested from human placenta is used as an adjunct, as a substrate for epithelial growth and ocular surface reconstruction [56]. Therapy using limbal stem cells has a reasonable success rate even in patients with severely diseased cornea.

The role of bone marrow stem cells as a source of ocular surface tissue is yet to be evaluated.

Retinal transplantation as therapy for retinal degenerative diseases such as retinosa pigmentosa (RA) and glaucoma has gained interest during the past 20 years. Structurally, retina is organized into three cellular layers: photoreceptor, interneuron and ganglion cell layers. RA is characterized by the widespread degeneration of the rods and cones in the photoreceptor layer. In glaucoma, ganglion neurons are the major targets of degeneration. Hence cell-based therapies for these degenerative diseases are directed towards replacing the missing neurons with new ones, thereby hoping to restore vision. Since retina to some extent is an immunologically privileged site, allogenic transplantation is highly feasible. Various types of cells and tissues are being investigated for treating retinal regeneration (table 1). Transplantation of retinal pigment epithelial (RPE) cells in animal models of RPE degeneration has been reported to improve photoreceptor survival and visual outcome. Some clinical benefits have been observed in patients with macular degeneration after autologous transplants of RPE to the fovea [57]. Embryonic retinal progenitor cells are not easy to be maintained

indefinitely in culture, and there are additional issues related to tissue availability and reproducibility [57,58]. Adult neural stem cells from the hippocampus have also been reported to incorporate into the retina and adopt the morphologies and positions of bipolar, horizontal, photoreceptor, and astroglial cells [59].

A breakthrough has been achieved with the discovery of retinal stem cell (RSC) population from human adult ciliary epithelium [60]. These RSCs can be expanded from single cells and differentiate into a variety of retinal cell types. Human RSCs have also been shown to integrate and differentiate into photoreceptors after transplantation into the host neonatal retina [61]. However strategies for *in vitro* expansion of RSCs and photoreceptor development still need to be optimized.

Recent studies demonstrate that hESCs can also be induced to generate retinal progenitor cells, which in culture can differentiate into photoreceptors [62,63].

### 7.3 Cardiovascular

Cardiovascular diseases are one of the leading causes of death and disability worldwide. These diseases lead to loss of cardiac tissue through death of the cells by apoptosis and necrosis. The average left ventricle contains approximately 4 billion cardiomyocytes and the myocyte deficit in infarction-induced heart failure is about one billion cardiomyocytes [64]. The remaining myocytes are unable to reconstitute the host tissue, and the diseased heart deteriorates functionally with time. Current therapeutic approaches available including medical therapy, mechanical left ventricular assist devices, and cardiac transplantation are primarily focused at limiting disease progression rather than repair and restoration of healthy tissue and function. The limited efficacy and comorbidity of these current treatments have thus increased the interest to investigate other alternative and additional long-term therapeutic strategies. In this context, a cell-based therapy for myocardial regeneration seems to be a potentially beneficial approach to achieve cardiac repair.

Several cell types that might replace necrotic tissue and minimize scarring have been considered (table 1). Initial cardiac cell transplantation efforts have utilized skeletal myoblasts (SMBs), adult stem cells isolated from skeletal muscle biopsies [65]. Based on their utility in animal studies, SMBs have been utilized in several clinical trials in patients with severe post-infarction left ventricular dysfunction [66–68]. Follow-up studies have shown a moderate, but significant increase in the left ventricular ejection fraction (LVEF), as measured by echocardiography. Similar to SMBs, an

improvement in cardiac function has also been observed in rats after coronary artery ligation followed by transplantation of fetal cardiomyocytes as compared to non-engrafted infarcted hearts [69].

In the bone marrow, three populations of stem cells: HSCs, MSCs and endothelial progenitor cells (EPCs) have been reported to contribute to heart muscle repair. In animal models of heart disease, administration of bone marrow derived stem cells has shown to cause an increase in tissue perfusion, a reduction in apoptosis, reduction in infarct size, and improvements in global and regional cardiac function [70,71]. The first randomized trial called BOOST trial (bone marrow transfer to enhance ST-elevation infarct regeneration) was performed by Helmut Drexler's group in Hannover, Germany [72]. The study demonstrated that intracoronary transfer of autologous bone marrow cells 4.8 days after percutaneous coronary intervention enhanced LVEF primarily in myocardial segments adjacent to the infarcted area. Another multicenter trial (reinfusion of enriched progenitor cells and infarct remodeling in acute myocardial infarction), REPAIR-AMI showed that compared to placebo treatment, intracoronary infusion of bone marrow cells resulted in improved left ventricular function at 4 months and reduction in combined clinical end points of death, recurrence of AMI, and any revascularization procedure at 1 year [73]. The benefit was greatest in patients with poor left ventricular function. However, other groups from Belgium and Norway, had been unable to detect a difference in outcome between bone marrow cell treated group and controls in AMI setting [74,75]. Different cell isolation protocols as well as dosage, degree of cell viability and function prior to delivery may contribute to the heterogeneous clinical results in randomized trials. In the (transplantation of progenitor cells and recovery of LV function in patients with chronic ischemic heart disease) TOPCARE-CHD trial, the absolute change in LVEF at 3 months, was significantly greater among patients receiving the bone marrow cells than among those receiving circulating progenitor cells [76]. An alternative approach used includes the mobilization of endogenous stem or progenitor cells *in vivo* from the bone marrow, to the damaged heart using specific cytokines and growth factors. Recent meta-analysis including 8 randomized controlled trials has demonstrated that, granulocyte-colony stimulating factor therapy increased LVEF by 1.09% in patients with AMI [77].

There is now accumulating evidence that the heart itself contains resident stem cells with the capacity to differentiate into cardiac myocytes [78]. In humans, autologous cardiac stem cells can be isolated from surgical or endomyocardial biopsies

and clonally expanded *in vitro* [79]. Human cardiosphere-derived cells (CDCs) when injected into the border zone of myocardial infarcts engraft and migrate into the infarct zone. Injected CDCs have also been shown to result in an increased percentage of viable myocardium and improve LVEF [80]. However, lack of sufficient numbers of cells that can be isolated from biopsies from patients hinders the clinical utility of cardiac stem cells.

Exciting new advances in cardiomyocyte regeneration are also being made in human embryonic stem cell research. Studies by Itskovitz-Eldor *et al* and Kehat *et al* have shown that hESCs can reproducibly differentiate in culture into embryoid bodies and the cells have structural and functional properties of early stage cardiomyocytes [9,81]. However, if hESCs are to have a future in cell-based cardiac repair, substantial improvement in the efficiency by which cardiomyocytes can be generated from hESCs has to be achieved. Until quite recently, the typical method for obtaining hESC-CMs was to form embryoid bodies (in medium including a relatively high fraction of fetal calf serum) and then harvest the resultant spontaneously contractile cardiomyocytes by either mechanical dissection [9] or enzymatic methods [82]. Embryoid bodies contain an admixture of many differentiated cell types, and so cardiogenesis is inefficient through this approach. Recent efforts are directed at identifying defined factors to enhance the differentiation of cardiomyocytes from hESC [82,83]. Nonetheless, in experimental studies, the transplantation of mESC-derived cardiomyocytes into the uninjured hearts of immunocompatible mice has resulted in the formation of stable intracardiac grafts [84–86]. In 2004, Kehat *et al* reported the first human cardiomyocyte transplantation into the uninjured swine myocardium [87]. Since then, transplantation of ESC-derived cardiomyocytes into normal and injured heart in animals has been shown to improve the global myocardial function, although for a short period of time [88,89].

Besides cardiomyocytes, two other cell types that are important to a properly functioning heart are the vascular endothelial cells, which forms the inner lining of new blood vessels, and the smooth muscle cell, which forms the wall of blood vessels. The heart has a large demand for blood flow and these specialized cells are important for developing a new network of arteries to bring nutrients and oxygen to the cardiomyocytes after heart tissue has been damaged. The potential capability of both embryonic and adult stem cells to develop into these cell types is also being explored as part of a strategy to restore cardiovascular function *via* the processes of therapeutic angiogenesis and arteriogenesis. In this

regard, bone-marrow derived EPCs isolated from peripheral blood and/or bone marrow have shown incorporation into sites of physiological and pathological neovascularization in the endothelium after either systemic injection or direct intramyocardial transplantation in animal models of peripheral limb ischemia and myocardial infarction [90–92]. Several clinical studies have however reported an inverse correlation between the number and activity of circulating EPCs and risk factors for coronary artery disease [93,94]. In this regard, genetic engineering of EPCs with growth factors offers a useful approach to developing these cells into efficient therapeutic tools. Iwaguro *et al* have demonstrated that the transfer of VEGF in *ex vivo* expanded EPCs enhances EPC proliferation, adhesion, and impaired neovascularization in an animal model of experimentally induced limb ischemia [95]. Gene modified EPCs have also been shown to serve as cellular vehicles for the delivery of therapeutic genes such as eNOS to the reconstituted endothelium [96]. The use of autologous EPCs seeded onto a scaffold has also been reported for the tissue engineering of heart valves [97,98]. hESCs have also been demonstrated to differentiate into EPCs and then to mature endothelial cells leading to vascular network structures in three dimensional culture models [99].

A final issue worth considering is the mechanism by which the implanted cells mediate the beneficial effects on contractile function. Several lines of evidence support the concept that new endogenous or exogenous cells can incorporate and become functional within the heart. Early studies with bone marrow derived HSCs in mice have suggested that they differentiate into cardiomyocytes after transplantation to induce the repair of damaged myocardium [71]. However, more recent studies with genetically marked cells indicate that the transplanted cells do not transdifferentiate into cardiomyocytes [100,101]. It is possible that the stem cells confer their beneficial effects, possibly by secreting paracrine factors that are cardioprotective or angiogenic.

Cellular cardiomyoplasty, although appears promising in pre-clinical studies, its safety and efficacy have not been adequately evaluated. Its future will depend on conducting carefully controlled, randomized clinical trials with appropriate selection of end points. Controversies exist over the specific cells to be used, the dosages needed for tissue repair, route of administration and how the transplanted cells would affect the electrical activity of the myocardium. Whether the cells can improve myocardial function after transplantation over long term is also not yet clear.

The challenge in regenerative therapy in cardiac diseases is not simply to arrest cardiac dysfunction

but to achieve cell engraftment with electro-mechanical integration into the heart, arrest adverse myocardial remodeling and improve contractility of the diseased heart.

#### 7.4 Neurological

Despite the protection of the central nervous system (CNS) by the skull and vertebral column, it remains susceptible to several insults and neurodegenerative diseases. The hallmark of several degenerative disorders in the CNS such as amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and Alzheimer's disease (AD) is the massive loss of one or several types of neuronal populations. There is evidence both in humans and in experimental animal models of neurodegenerative diseases for spontaneous neurogenesis involving endogenous neural stem cells (NSCs) [102–105]. This putative endogenous repair process appears to be insufficient to compensate neuronal loss and to ensure functional recovery. These observations have raised interest in the use of exogenous embryonic and adult stem cells in substitution therapies with the hope that these cells could generate new neurons after they are grafted into lesioned nervous tissues [106]. Stem cells used for applications in neurological diseases are from four different sources: NSCs from the embryonic or the adult brain, stem cells from other tissues such as the bone marrow and ESCs (table 1).

Adult NSCs exist within multiple regions of the CNS (subventricular zone, hippocampus, etc.), and it is possible to isolate and expand these cells to give rise to progenitor cells restricted to defined neural lineages such as neuronal and glial cells [107]. Neural stem cells that proliferate in the ventricular region and later in the subventricular zone of the developing brain give rise to three neural lineages of the CNS, i.e., neurons, astrocytes, and oligodendrocytes [108]. The identification of NSCs and progenitor cells has completely challenged the past notion that adult brain is an organ with no ability for self-renewal.

Demyelinating diseases of the brain are attractive targets for cell-based therapeutic strategies, since these diseases are caused by the loss of a single cell type, the oligodendrocyte. In experimental models of focal demyelination in rodents, it has been shown that endogenous cells in the CNS have the potential for regenerating oligodendrocytes and myelin [109,110]. Injection of adult NSCs has been demonstrated to induce recovery in a chronic model of multiple sclerosis [111,112]. Transplantation of NSCs of various origins has also resulted in the improvement of clinical outcome in experimental models of spinal cord trauma and the

therapy improves myelinating properties as well [113–115].

Neurodegenerative diseases such as PD disease involve continuous loss of dopaminergic neurons. Stem cell therapy for PD is aimed at the induction and renewal of dopaminergic neurons. In neurodegeneration models of PD, *ex vivo* expanded NSCs efficiently decrease parkinsonian symptoms by rescuing dopaminergic neurons through production of specific growth factors [116,117]. Likewise, transplantation of NSCs into the lumbar spinal cord of rodents with ALS has been shown to postpone the disease onset, to preserve the viability of motor neurons, and to prolong animal survival [118,119].

Alteration in the local blood flow is believed to participate in the progression of neuronal death after stroke. Accumulating evidence suggests that after transplantation, NSCs migrate toward ischemic boundary regions in embolic stroke and that the engrafted cells increase angiogenesis [120].

In addition to adult NSCs, two prototypic stem cell populations from the adult bone marrow, *viz.*, HSCs and MSCs can also transdifferentiate into neural cells. Indeed, both cell types have been shown to migrate efficiently towards the site of injury within the CNS [121]. Mimicking NSCs, MSCs also promote functional recovery after brain injury in several experimental models [122–125]. For example, it has been shown that intraventricular as well as intravenous transplantation of MSCs into mice with multiple sclerosis, result in significant clinical improvement [126]. Similarly, cerebral neovascularization, restoration of cerebral blood flow, and reconstitution of the blood–brain barrier in animal models of stroke have been obtained with HSCs [127–129] and MSCs through enhanced production of VEGF [130] and FGF-1 [131]. Further, *in vivo* experiments suggest that MSCs can induce the proliferation of endogenous NSCs [132] and their differentiation into oligodendrocytes [133] or astrocytes [134].

Besides adult stem cells, studies have shown that undifferentiated ESCs grafted into lesioned brain develop into normal dopaminergic neurons and express neuronal and dopaminergic markers *in vivo* [10,135]. Nevertheless, despite the promising results obtained with ESCs in experimental models of nervous insults [136,137], the risk of transplanted cells evolving into teratomas [135] combined with ethical issues limit the use of ESCs in cellular therapies.

Recently, continuously dividing immortalized cell lines of NSCs have been generated by the introduction of oncogenes. These immortalized NSC lines have emerged as a highly efficient source of cells for genetic manipulation and gene transfer into the CNS *ex vivo*. Once transplanted into the

damaged brain, these cells survive well, integrate into host tissues, and differentiate into both neurons and glial cells [138–144].

Of the various cell types, NSCs have the most potential for use for treating the broad spectrum of neurological disorders. However before embarking into routine clinical use, further studies are warranted to identify the signals for proliferation, differentiation, and integration of NSCs and also to determine the environment conditions of the host brain favorable for implanted NSCs to survive, prosper, and restore damaged tissue.

### 7.5 Musculoskeletal

Since the pathbreaking studies of Friedenstein *et al* who isolated bone-forming progenitor cells from rat marrow, the ability of these cells, designated the MSCs to differentiate into various cell types of mesenchymal tissues, including cartilage, bone, fat, muscle, tendon, has been widely recognized. Although MSCs represent only a very small fraction of the total population of nucleated cells in the marrow, they can be easily isolated and extensively expanded or specifically differentiated under appropriate *in vitro* conditions. Besides the bone marrow, the multipotential MSCs for bone regeneration have also been isolated from other sources such as the adipose tissue and skeletal muscle. An added advantage of using MSCs is that they do not elicit alloreactive lymphocyte proliferation and modulate the immune responses [145,146].

The ability of MSCs to form bone was one among the first properties to be evaluated. In animal models of bone defects, implantation of MSCs adsorbed onto appropriate scaffolds, resulted in a significant increase in the rate of bone formation and also improvement in the physical properties of the bone [147–149]. Success in animal studies paved way for initiation of the first clinical trial. Quarto *et al* reported repair of large segmental defects in humans using autologous MSCs on hydroxyapatite scaffolds [150]. In animal models, MSCs delay graft rejection, and in children with osteogenesis imperfecta, allogenic bone marrow transplantation result in the engraftment of donor derived MSCs and new bone formation [151]. Recently, genetically engineered MSCs with potent osteogenic genes such as BMPs have been used to repair fracture repair and rapid bone formation has been observed *in vivo* [152]. MSCs have also been evaluated as a substitute for chondrocytes in the cartilage repair process. In a few animal studies, implantation of MSCs has been seen to differentiate both into cartilage and subchondral bone [153,154]. Ongoing investigations have now been focusing to engineer MSCs into soft tissues, tendons and ligaments that play a major role in the movement of joints.

Besides MSCs, autologous articular chondrocytes have also been in use for local cartilage repair in both animal and clinical studies.

Stem cell therapy to repair and replace damaged skeletal muscle cells in chronic, debilitating muscle diseases such as muscular dystrophies has shown great promise. Different stem cell populations, both of embryonic and adult origins appear to have the potential to generate skeletal muscle cells and have been studied in animal models of muscular dystrophy (table 1). Caplan and colleagues first investigated *in vitro* differentiation of bone marrow derived MSCs into muscle [155]. More recently, Cossu, Mavillo and co-workers have demonstrated active muscle regeneration *in vivo* with bone marrow-derived cells [156]. Several stem cell populations have recently been recognized in skeletal muscle [157,158]. Satellite cells are dormant progenitors often referred to as 'muscle stem cells' and located beneath the basal lamina of mature skeletal muscle fibers. These cells are considered to be monopotent stem cells capable of giving rise only to cells of the myogenic lineage. Among other progenitor cells found in skeletal muscle are side-population (SP) cells, mesoangioblasts, and pericytes [159]. SP cells have a tremendous ability to proliferate and provide myoblasts for muscle regeneration. They also appear to be able to differentiate into additional lineages [160]. Gussoni and colleagues demonstrated the restoration of dystrophin expression in the mdx mouse (an animal model of Duchenne muscular dystrophy) by using SP population from donor marrow [161]. The inherent vascularity of the muscle makes it a useful depot to deliver secreted proteins *via* gene therapy. Genetically engineered myoblasts, or muscle-derived stem cells, have been used for replacing degenerating muscle in Duchenne Muscular Dystrophy [162,163] or in bone defects [164]. As a gene delivery vehicle, myoblasts have been employed to deliver growth hormone, VEGF, Factor IX, erythropoietin and several other molecules [165–168].

The myogenic potential of ESCs has been well demonstrated in the *in vitro* models [169]. A recent study has reported the transformation of hESCs into satellite-like myogenic stem cells with remarkably high engraftment efficiency compared to myoblast transplantation in a muscle injury model [170]. Levenberg *et al* have described a method for the *in vitro* expansion of engineered skeletal muscle tissue developed by means of co-seeding the myoblasts with hESC-derived endothelial cells and embryonic fibroblasts on a porous biodegradable scaffold. The co-culture of myoblasts in the presence of hESC-derived endothelial cells resulted in neovascularization in the construct prior to implantation,

which contributed to improved integration of the engineered muscle when transplanted to immunodeficient mice [171].

The potential for stem cell regeneration of musculoskeletal tissues seems immense. One of the major challenges of any orthopedic application would be to identify the proper biocompatible matrix, one that will withstand the immediate structural forces, provide for cell differentiation along appropriate lineage paths and be resorbed at rates proportional to the rate of increase in strength of the newly formed matrix.

## 7.6 Renal

The kidney has a remarkable capacity to regenerate after injury, as it is not a terminally differentiated organ. This regenerative potential is somehow incomplete and as the insult continues progressive and irreversible scarring results in chronic renal disease. End stage renal disease is a deadly disease unless supportive treatment is given in the form of hemodialysis, peritoneal dialysis or kidney transplantation. An acute shortage of compatible organs, coupled with limited adaptability of current dialysis techniques has spurred a sense of urgency to investigate newer alternatives such as cell-therapy.

Three stem cell lineages of the bone marrow: HSCs, MSCs, and EPCs have the potential to promote repair in various forms of kidney disease (table 1). Bone marrow-derived stem cells seem to have a high capacity for transdifferentiation and therefore are able to replace damaged renal tissue with tubular epithelial cells, mesangial cells, endothelial cells, and even podocytes [172,173]. Injection of MSCs protects the kidney from toxin or ischemia/reperfusion injury and attenuates lost renal function, whereas injected HSC do not have the same effect [174]. The first phase of clinical trials using bone marrow MSCs for protection against acute kidney injury may begin shortly. This study hopefully would enable further exploration of stem cell therapy in renal patients with multiple comorbidities.

Participation of circulating EPCs in renal endothelial repair has been demonstrated in several experimental studies [175,176]. Transplantation of *ex vivo* expanded EPCs from a muscle stem cell pool has shown to locally engraft, and improve renal function in rats with acute renal ischemia [177]. Animal studies have also provided evidence that EPCs contribute to glomerular capillary repair [178,179]. In the clinical setting, renal diseases in concert with cardiovascular risk factors have been reported to significantly influence the number and function of EPCs [180,181].

Multipotent resident renal stem cells have not yet been discovered in the kidney. However, Oliver

*et al* have demonstrated the existence of resident stem-cell pools in the renal papilla [182]. Iwatani and colleagues have suggested that renal stem cells may reside in the bone marrow and take up residence in the kidney when needed [183].

Whether human ESCs can be used as a starting material for renal regeneration still remains to be determined.

## 8. Stem cells and tissue engineering

Since stem cells are highly regulated by their microenvironment or the niche in which they reside, efforts are on to provide constructs that can mimic the cell milieu through development of tissue-engineered scaffolds [184]. These scaffolds also temporarily provide biomechanical support for cells until they are able to produce their own extra-cellular matrix [184]. Better control of the tissue formation process is an additional advantage. Scaffolds are typically fabricated by natural materials, which are inherently bioactive but lack mechanical strength, or synthetic materials, which lack inherent bioactivity but could be mechanically strong and can be fabricated with the desirable macro- (shape) and microarchitecture (pore size, porosity). Numerous types of biomaterials both man-made or from natural sources are continually being discovered [185]. Efforts are being carried out to modify the surface of these materials, to guide, and enhance stem cell differentiation. Initially, scaffolds were designed to be bioinert. Currently, biomaterials are made to interact with the cells that release growth factors, genes, or other signals in a time-dependent manner [185–187]. Based on these active bio-materials, the conventional two-dimensional (2-D) culture models have now paved the way for three-dimensional (3-D) culture environments that mimic the *in vivo* environments more closely and hence are more conducive to regulating stem cell proliferation and differentiation [188]. Elements of the extracellular matrix and stromal MSCs have gained increasing attention as potentially crucial mediators in developing and maintaining the characteristics of 3-D cell cultures. Fibrin alone or in combination with other materials has emerged as an important biological scaffold for stem cells to regenerate adipose tissue, bone, cardiac tissue, cartilage, liver, nervous tissue, ocular tissue, skin, tendons, and ligaments [189]. Culture on fibrous biodegradable scaffolds that mimic basement membrane texture has resulted in an increased expansion of both HSCs and ESCs [184]. Similarly, the immobilization of cell-associated Notch ligands has shown to increase the self-renewal of HSCs [190]. A perfect tissue engineered scaffold is elusive at present. The scaffold

should not only support attachment, spreading growth and differentiation of cells but also control inflammation and foreign body reaction. It should be biodegradable into non-toxic products, sterilizable and manufacturable. It should offer options to deliver drugs, cytokines and genes. The set of criteria would appear demanding, but has to be met for the tissue-engineered scaffolds to be effective.

## 9. Stem cell research in India

Stem cell research has gained considerable impetus in India in the recent years. Draft guidelines for stem cell research in the country have been formulated jointly by the Department of Biotechnology and Indian Council for Medical Research. Several groups are actively and enthusiastically pursuing the field with reasonably good results. According to a recent review, for haematological disorders, a total of 1540 bone marrow transplants have been performed in a country of over one billion population [191]. At Christian Medical College (CMC), in Vellore, a total of 626 transplants have been performed in 595 patients, with 28 patients having more than one transplant from October 1986 to December 2006 [191]. Besides, CMC Vellore, autologous and allogenic bone marrow or blood stem cell transplantation is being performed at other hospitals such as All India Institute of Medical Sciences (AIIMS), New Delhi and Tata Memorial Hospital, Mumbai [192–194]. AIIMS has also set up the country's first cord blood bank for isolation of cord blood stem cells for in-house patients. At the L V Prasad Eye Institute, Hyderabad, transplantation of autologous cultivated limbal stem cells in patients with limbal stem cell deficiency, has shown a successful outcome with a stable ocular surface without conjunctivalization [195]. Small scale phase-I clinical trials using bone marrow stem cells have been reported for the treatment of diabetes at Dr. H L Trivedi Institute of Transplantation Sciences, Ahmedabad [196], acute myocardial infarction at Nizam's Institute of Medical Sciences, Hyderabad [197], Sir H N Hospital and Research Centre, Mumbai [198] and nonischemic dilated cardiomyopathy at AIIMS, New Delhi [199]. At Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum, procedures for the isolation and expansion of EPCs from peripheral blood of patients with CAD have been optimized [200]. Recent strategies are now directed towards augmenting the angiogenic potency of these cells by modulation with endothelial nitric oxide synthase gene transfer. Besides EPCs, ckit-positive stem cells have been isolated from atrial

biopsies of CAD patients and also induced to differentiate into beating cardiospheres [201]. At the biomedical technology wing of SCTIMST, recent studies have reported that platelet rich plasma in combination with goat bone marrow-derived MSCs cultured on bioactive ceramic scaffolds leads to a much faster sequence of healing events in large segmental bone defects in a goat femur model [202]. Stem cell research at the Centre for Cellular and Molecular Biology, Hyderabad has been focusing on the genetic and epigenetic mechanisms governing the transient dormancy and activation of satellite cells, the stem cells in adult muscle tissues [203,204].

Vanikar *et al* have reported the generation of 30 healthy hESC lines from 33 voluntary oocyte donors using a donor somatic cell nuclear transfer technique on 190 oocytes [205]. Researchers at National Brain Research Centre, Gurgaon and National Centre of Cell Sciences, Pune are working towards the differentiation of hESCs into neural stem cells [206–208]. Very recently, Jagatha *et al* have demonstrated the potential of FGF2-induced ES cell derived neural progenitors (ES-NPs) to generate retinal ganglion-like cells *in vitro* upon differentiation [209]. At the Reliance Life Sciences, Mumbai, functional dopaminergic precursor neurons from human embryonic stem cells (hESCs) have been recently reported. Transplantation of these precursor neurons into the lesioned rat model of Parkinson's disease has also shown to elicit significant reversal of lesion induced motor deficits sustained up to the end of 1 year long study period [210]. Researchers at the Reliance Life Sciences have also demonstrated the generation of spontaneously beating cardiomyocytes using FGF from ESCs [211]. Studies at the Manipal Institute of Regenerative Medicine, Bangalore are directed towards the optimization of culture conditions of human MSCs with an attempt to obtain large numbers, preserve their characteristics and multilineage differentiation potential for therapeutic uses [212]. They have also reported the derivation of FGF2 expressing germ layer derived fibroblast cells from hESC lines for use as a feeder layer for culture of hESCs. These feeders could support the pluripotency, karyotypes and proliferation of hESCs with or without FGF2 in prolonged cultures as efficiently as that on mouse embryonic fibroblasts [213].

## 10. Current challenges and future possibilities

Besides the overwhelming promise of stem cells in various cellular therapies, their clinical and practical use is constrained by several technical and

ethical issues. The biggest hurdle for the clinical use of adult stem cells is the small number of cells that can be isolated from any adult tissue. The identification of cells and factors in the so called 'stem cell niche' affecting the growth and differentiation of resident adult stem cells may be one possible answer. For example, the bone marrow stromal cells are known to promote proliferation and differentiation of HSCs in long-term cultures [214]. The other approach is based on introduction of genes in the supporting feeder layer of cells that inhibits differentiation of target cells. The up-regulation of notch ligands such as Jagged-1 and Delta in the stromal cells by gene modification strategies has been demonstrated to promote the expansion of stem cells without inducing differentiation [26,27,190]. Another technique actively pursued is the usage of modified stem cells. Based on our understanding of the molecular pathways responsible for self-renewal and proliferation of stem cells as well as discoveries of new genes that control stem cell proliferation and differentiation, novel strategies have come up. For example, *HOX* genes that are expressed during early development and which govern various processes including body-part patterning have been shown to increase the self-renewal potential of HSCs [215].

Destruction of life in the form of an embryo has been a major ethical objection in embryonic stem cell derivation and research in several western countries. One way that has been suggested to circumvent the objection is to fuse existing hESCs with an adult somatic cell, generating a cell line that retains ESC specific properties and yet has the genotype of the somatic cell [216]. There is however no technology available at present to selectively remove all the ESC chromosomes while retaining the somatic cell chromosomes. Development of such a technology is potentially expensive and will presumably take many more years. Other approach is the generation of induced pluripotent cell lines from induced somatic cell dedifferentiation. In this method, the adult somatic cells are genetically modified and reprogrammed to undergo a process of dedifferentiation [22].

Availability of methods for growth and maintenance of ESC in culture present another major obstacle to their potential clinical use. Conventionally, hESC lines are grown in a medium containing animal serum as a source of nutrients and growth factors and then on mouse-derived fibroblast as feeder layers. The use of any cell based therapeutic agent in humans must however be free of animal contamination. In this direction, some laboratories have successfully cultured hESCs in a serum-free defined medium on human cell-derived feeders or even in feeder free conditions [217,218].

The risk of tumor formation following transplantation of hESC is another factor to be considered. Studies with both ESCs and ES derived differentiated cells have shown that they can form teratocarcinomas in adult mice if injected subcutaneously, intramuscularly or into the testis [219,220]. The suitability of ESCs for transplantation purpose has also been skeptical because of the observed genetic instability of cloned cells and extreme inefficiency of the process [221]. Allergrucci *et al* recently reported that hESCs could undergo epigenetic changes over time in culture [222]. All these observations indicate the need for optimization of procedures and periodic monitoring of the cell lines to ensure their genetic stability and hence suitability for *in vivo* applications.

Finally, immunological issues are a major concern for allogenic stem cell transplantations with both adult and embryonic stem cells from non-autologous sources. Rejection can be inhibited by the use of immunosuppressive drugs, which can have serious side effects. Technologies to develop individual-specific stem-cell lines through somatic-cell nuclear transfer or cell fusion may allow engineered stem cells containing the individual's own genetic material to be used for treatment [223]. The development of a bank of MHC-compatible hSC lines is also a lucrative option, though it also carries with it several ethical and technical problems. Another possible way to overcome immune rejection is to over express into the stem cells, genes such as fas-ligand that can suppress the immune system [224]. It has also been suggested that elimination of certain immunologically reactive cell surface molecules like B7 antigens or CD40 ligands from the stem cells prior to transplantation could also contain the immune rejections to some extent [225].

## 11. Conclusion

A silhouette of the potential use of stem cells for treatment of human disease is now perceptible. The coming years will undoubtedly usher in new developments and technologies that would translate the envisioned therapeutic potential of stem cells to bedside medicine for patients suffering from devastating and debilitating diseases. The challenge in stem cell therapy is not simply to arrest organ dysfunction but is to achieve cell engraftment with functional integration into the organ, arrest adverse tissue remodeling and improve function of the diseased organ. To understand underlying mechanisms and to answer the many unknown questions related to regenerative therapy requires the knowledge and expertise of many disciplines.

Be that as it may, stem cell therapy for regeneration has undoubtedly arrived.

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