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Stem Cell Based Tissue Engineering and Regenerative Medicine: A Review Focusing on Adult Stem Cells

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Abstract

In the past 30 years, the field of tissue engineering has continued on a fast paced march. Early discoveries in embryonic stem cells fueled a wave of research that led to wild claims about regenerating nonfunctioning organs. Although we are still a far way away from being able to grow functional organs in a Petrei dish, the field continues to progress forward, and new clinical trials begin often that apply the use of stem cell based solutions for regenerative medicine and tissue engineering. Current trends have focused more on adult stem cells for these tissue based therapies and regenerative medicine as they offer an autogenic source, and are less tumorigenic than their embryonic and induced-pluripotent stem cell counter parts. Current therapies in myocardial tissue repair, neural tissue repair, diabetes, osteogenic and chondrogenic differentiation are reviewed.

Classes of Stem Cells

Embryonic Stem Cells (ESC)

Derived from the inner cell mass of *in vitro* fertilized blastocyst-stage embryos prior to germ layer formation, ESC's are the cell population that all cells of the mature body are formed from. With appropriate culture conditions, including growth cocktails, co-culture with differentiated cells, and various other protocols, these cells have been shown to be pluripotent or capable of differentiating into cells from all three of the developmental germ layers, endoderm, mesoderm, and ectoderm. Human ESC's (hESC) were first isolated in 1998 by James Thomson, using *in vitro* fertilized embryos, and were shown to form teratomas, or tumors with all three germ layers present. [1]

Issues associated with teratoma formation, and immunogenicity concerns; however, have limited the clinical application of hESC's. Associated tumorigenisis with these cells is believed to be correlated to the length of culture of these cells, as it is immortal cells that are inherently selected for. Recent developments, however, have shown that by differentiating ESC's prior to implantation and sorting for undifferentiated cells, the risk of teratoma formation decreases. [2,3] This has led to the creation of two approved clinical trials that are currently under way. One trial is using ESC derived retinal pigmented epithelia (RPE) for transplantation in patients with Stargardt's Macular Degeneration, this trial was approved by the Food and Drug Administration in November of 2011, and still actively recruiting patients. [4] Other active clinical trials underway using ESC's are for spinal cord injury repair.

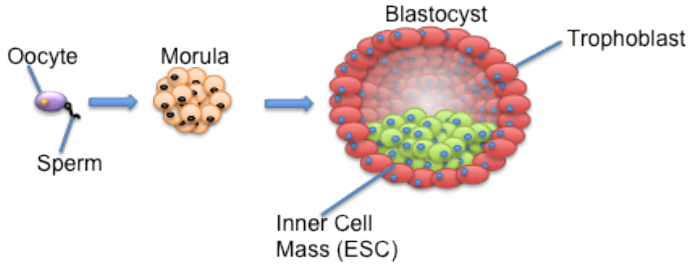

Figure 1-Derivation of Embryonic Stem Cells

In addition to the risk of tumorigenicity, questions regarding the immunogenicity have also been raised in accordance with the use of ESC's. Since these stem cells are allogeneic, they will inevitably pose a risk of host rejection post transplantation. Current standard of care practices for patients receiving allogeneic cell transplantation involves the use of immunosuppressive drugs to prevent overall immune

rejection, however this is imperfect solution as it makes the patient more susceptible to infection, and the previously mentioned tumor formation. [5]

Induced Pluripotent Stem (iPS) Cells

New research has shown that through induction of specific transcription factors and growing cells in conditions replicating the embryonic environment, somatic cells, or fully differentiated cells, can be reprogrammed to an embryonic like state. Such cells are called induced pluripotent stem (iPS) cells. Takahasi and Yamanaka first demonstrated the efficacy of genetic reprogramming of these cells in 2006. Results of this study showed that transfection of c-Myc, Sox2, Klf4, and Oct 3/4 genes are required for the genetic reprogramming of these cells, and removal of one of these genes does not lead to pluripotency in somatic cells. [6] Further studies by Thomson identified Nanog, Oct4, Sox2, and Lin28, as capable of reprogramming somatic cells to a pluripotent state. Significance of these results are that c-Myc had previously been shown to induce differentiation and apoptosis of hESC, in addition to being a known oncogene. [7] Both groups showed that these genetically reprogrammed cells possess the ability to differentiate into cells from all three embryonic germ layers, with some cells being able to accomplish this after 30 passages. These cells also exhibit the embryonic like morphology. It is now known that the only factor necessary for pluripotency is Oct4, and either Sox2 or Nanog. [8]

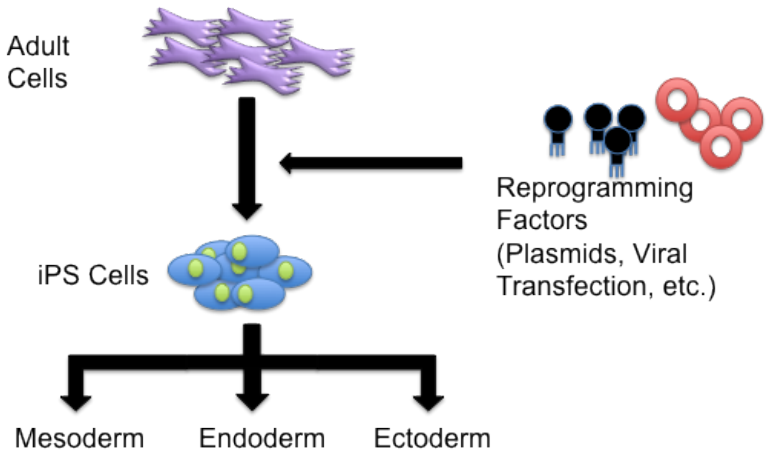

Figure 2-The generation of induced-Pluripotent Stem Cells

iPS cells are an ideal choice for tissue engineering as they are an autologous pluripotent cell source. However, there are several safety issues that must be addressed, most of which relate to the genetic reprogramming methods used to generate these cells which may lead to both genomic and epigenetic

issues. Recent studies have shown increased frequency of copy number variations (CNV) in iPS cells, as compared to their embryonic counterparts. This increase in CNV frequency leads to questions about the even greater potential of iPS cell lines to generate tumors. [9] Epigenetic studies of the DNA methylation have shown that iPS cells may also show some form of "epigenetic memory" in which DNA methylation patterns that are signatures of their somatic cell origin are present in the pluripotent cell and drive them towards specific lineages. [10] Other studies of epigenetics showed that methylation patterns could be aberrant and not related to the somatic cell, or embryonic cells. [11]

Adult Stem Cells (ASC)

To compensate for the normal tissue loss, it is believed that most adult tissues contain a cell type, known as adult stem cells, which are capable of generating new cells in these tissues. These cells exist throughout the body in regions known as "niches," and if these niches can be identified, and the resident stem cells identified, may be able to provide an autogenic source for stem cell transplantation. Mesenchymal stem cells (MSC) are a subpopulation of adult stem cells that have been located in bone marrow, adipose tissue, and Wharton's jelly of the umbilical cord. Over 120 clinical trials are registered with the National Institute of Health which applied MSC's for therapy, the majority of these trials are in phase I or II testing. [12] These trials include therapies running the gamut of Bone/Cartilage Disease, Heart Disease, Diabetes, GI Disease, and Neurodegenerative diseases. [13] Other categories of adult stem cells include: neural progenitor cells, which can differentiate into neurons, astrocytes, and oligodendricytes, and hematopoietic stem cells which give rise to the cellular elements of blood, to name a few. Potentially, if the niche for all adult stem cells could be isolated, and the resident stem cells purified, a regenerative cell source for all tissues of the body would exist. Due to the large variety of adult stem cells present in the body, we will choose to focus on each of these categories in the following subsections as they relate to each tissue type.

Current Stem Cell based Tissue Engineering Therapies

Myocardial Regeneration

According to the World Health Organization, 12.8% of deaths in 2008 were caused by ischemic heart disease, making it the leading cause of death worldwide. [14] The progression of ischemic heart disease deprives myocardial cells of oxygen, and places additional stress on existing cardiomyocytes

ultimately leading to heart failure. Current therapies are unable to reverse the effects of ischemic heart disease, and only act to slow the deterioration of existing myocardial cells. Currently, several cell types have been proposed as candidates for cardiomyocyte and endothelial cell regeneration, including ESC's, resident cardiac stem cells, and the previously noted mesenchymal stem cells. [15]

Several studies have shown that embryonic stem cells injected into the myocardium post infarct were able to engraft and differentiate to regenerate the three cell types found in the myocardium: myocytes, smooth muscle cells, and endothelial cells, this is known as tri-lineage differentiation. Inefficient differentiation and insufficient purity of the cells, as well as poor survival of transplanted cells hindered these early studies of ESC repair post-infarct. Recently, however, by pre-differentiating human ESC's into cardiomyocytes using Activin A, and BMP4, followed by a selection for cardiomyocytes, researchers were able to show that when transplanted into in infarcted heart in a mouse model showed partial remuscularization at the infarct site, and integration into the host myocardium. Without differentiation, injected cells showed teratoma formation in the host myocardium. To counter the poor survival cells a pro-survival cocktail is administered in concert with the cells. [16]Although the long-term goal of these cell-based therapies is the regeneration of lost myocardial tissues, not all stem cell based therapies result in direct differentiation of the stem cells into resident cardiac tissues. In a study of the perfusion of MSCs into porcine models three days post infarct, Hare reports that paracrine effects also play a key role in the regeneration of necrotic

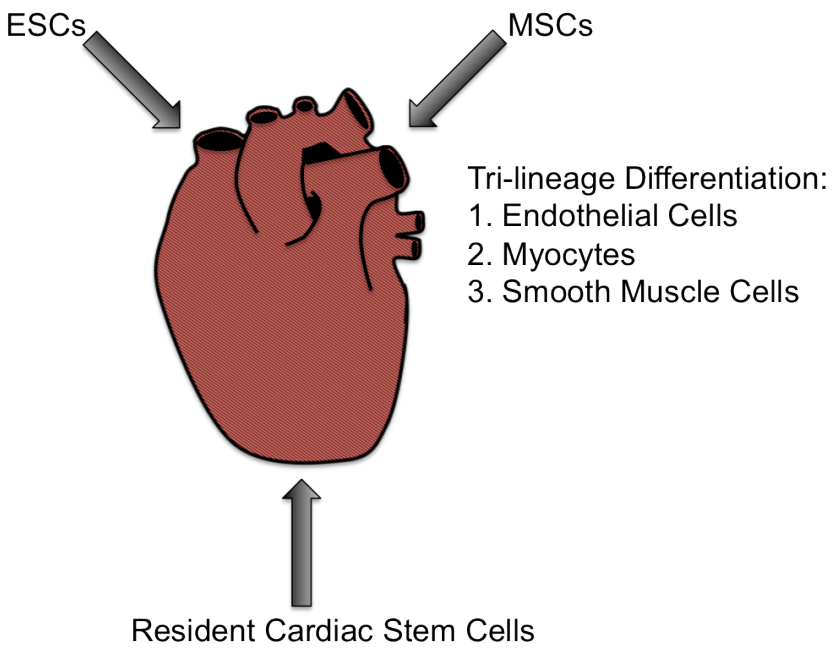

Figure 3-Stem cell repair for myocardial tissue

myocardial tissue. In this study Hare demonstrates an overall decrease in infarct size and increase in global cardiac function based on an increase in left ventricular ejection fraction, however low engraftment of the MSCs is also noted. The author suggests that the MSCs injected act as paracrine regulators for the native cardiac stem cells mentioned in the next section of this article, as well as secrete additional paracrine factors such as vascular endothelial growth factor (VEGF) to promote endogenous repair of the myocardium. [17] Because of the clinical nature of this study, labeling of injected cells was not possible; however, pervious studies showed that 20% of the administered cells were still present in the myocardium two weeks post-implantation [18] Further studies into the action of these MSC's suggest that they recruit the mobilization of bone marrow progenitor cells (BMPC), as well induces the existing myocytes to enter a proliferative stage regenerating the myocardial tissue. [19]

Initially it was thought that myocytes are unable to replicate, and thus that the adult heart has no potential to promote endogenous repair. However, recent studies have also shown that the adult human heart contains a population of resident cardiac stem cells that are able to regenerate myocytes post-cardiac injury. These cells are identified by the expression of cKit, and have been shown to be multipotent by differentiating into myocytes, endothelial cells, and smooth muscle cells. Upon transplantation of these cells to an infarcted mouse model, human myocardial growth was demonstrated in the infarcted region, generating a chimeric heart. [20]

Neural Differentiation

Diseases that affect deterioration of neural tissue such as Parkinson's disease, Huntington's disease, and Alzheimer's disease, are almost invariably fatal, and currently, the treatments that exist aim to slow the progression of the disease and decrease associated symptoms, but do not regenerate the damaged tissue. The complexity of the human brain, has provided many challenges associated with using stem cells for treatment regimens of these diseases. Since each of these diseases is specifically associated with loss of function of a distinct spectrum of neuronal tissue, the appropriate cell source is necessitated by disease pathophysiology, thus therapies for each of these diseases should be evaluated independently. [21]

Parkinson's disease is characterized by the loss of dopamine-synthesizing neurons from the midbrain region. A large fraction of the current research occurring for the treatment of Parkinson's disease

has focused on the use of iPS cells and ESC's. Chung et al. report that ESC's could be differentiated into dopaminergic neural progenitor cells. These cell lines showed stability for up to four weeks *in vitro* without demonstrating significant phenotypic aberrations. Transplantation of these cells into animal models of Parkinson's disease demonstrated improvement in the motor response of the animals, and histological evaluations showed well-integrated dopaminergic neural regions in the brain. [22]

Huntington's disease is an autosomal dominant linked disease that causes an expansion of CAG nucleotides in the gene coding for the protein Huntingtin, this in turn leads to deterioration of striatum region of the brain. MSC's have been proposed as a potential source for the neuronal degradation characteristic of Huntington's. Rat models that had been induced with Huntington's disease using quinolinic acid (QA) were transplanted one-week post QA administration. The effect of the MSC's transplantation was assessed on several measures including behavioral assessment, and striatum volume. Behavioral assessment and motor control, which is characteristically lost in patients with Huntington's disease, showed increased functionality two weeks post transplantation of the MSC's, and continued to increase for a period of eight weeks following the transplantation. Volume of the striatum region also was significantly larger in the MSC treated animals compared with the control. [23] Not only does this study show the potential of stem cells to be used as a therapy for Huntington's disease, but this study also serves to show that MSC's have to potential to differentiate into neuronal tissues as well.

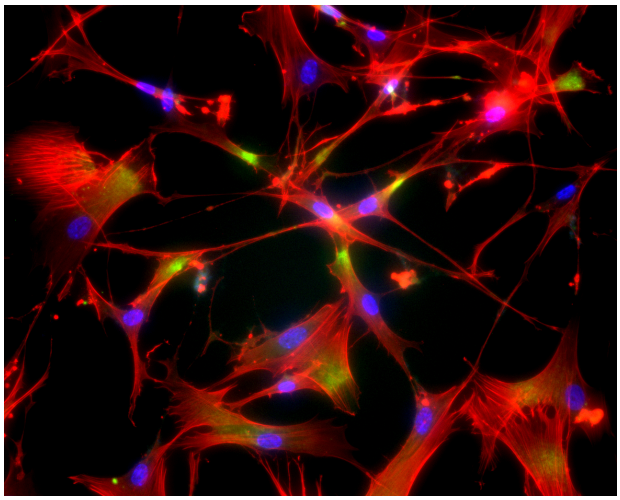

Figure 4-Neurons derived from peridontal ligament derived adult stem cells. Red stain is F-Actin, blue DAPI, and green Synaptophysin;
Photo courtesy of: Veronica Fortino

The seventh leading cause of death in the United Sates, and fifth for those over age 65 is Alzheimer's disease. Pathophysiology of Alzheimer's includes degeneration of synapses and neurons that function in learning and memory, specifically the hippocampus, entorhinal cortex, basal forebrain, and

neocortical association cortices. Brains of patients with Alzheimer's also display the presence of amyloid-beta peptide rich plaques, which is believed to cause the apoptosis characteristic of Alzheimer's disease. [24] In a recent study, Blurton-Jones et. al. studied the effects of neural stem cells on mice that demonstrated Alzheimer's symptoms. These researchers were able to show that post cell transplantation, the test animals regained their learning and memory abilities. Interestingly, however, their study showed no decrease in the amyloid-beta peptides present in the mice, but rather, they argue that the rescuing of these traits is due to increased hippocampal synaptic density which is caused by elevated levels of brain derived neurotropic factor (BDNF), released from the injected neural stem cells. Mice in these studies exhibited a 67% overall increase in synaptic density in the treated population versus the control. [25]

Diabetes and Stem Cells

Diabetes Mellitus is a metabolic disorder affecting the beta cells of the Islets of Langerhans in the pancreas. The islets are cell clusters which perform the endocrine functions of the pancreas, and are composed of three main cell types: alpha cells, which produce glucagon, beta cells, which produce insulin and delta cells, which secrete somatostatin. There are two classes of Diabetes Mellitus; Type 1 is characterized as an autoimmune disorder which leads to self-destruction of the individuals beta cells. Type II diabetes is characterized by a resistance to insulin as well as a decrease in insulin production. Both forms of Diabetes ultimately lead to the individual's inability to regulate blood glucose levels.

Individuals with Diabetes must monitor blood glucose levels, and perform insulin injections when necessary. Several therapies involving transplantation of functioning islets or beta-cells have been suggested and are currently undergoing trials. Current allogeneic therapies involve cadaveric transplantation, however two cadaveric sources are required to provide the amount of cells necessitated. A stem cell source that could produce functioning islets would be highly desirable, and help to restore glycemic control for individuals with diabetes.

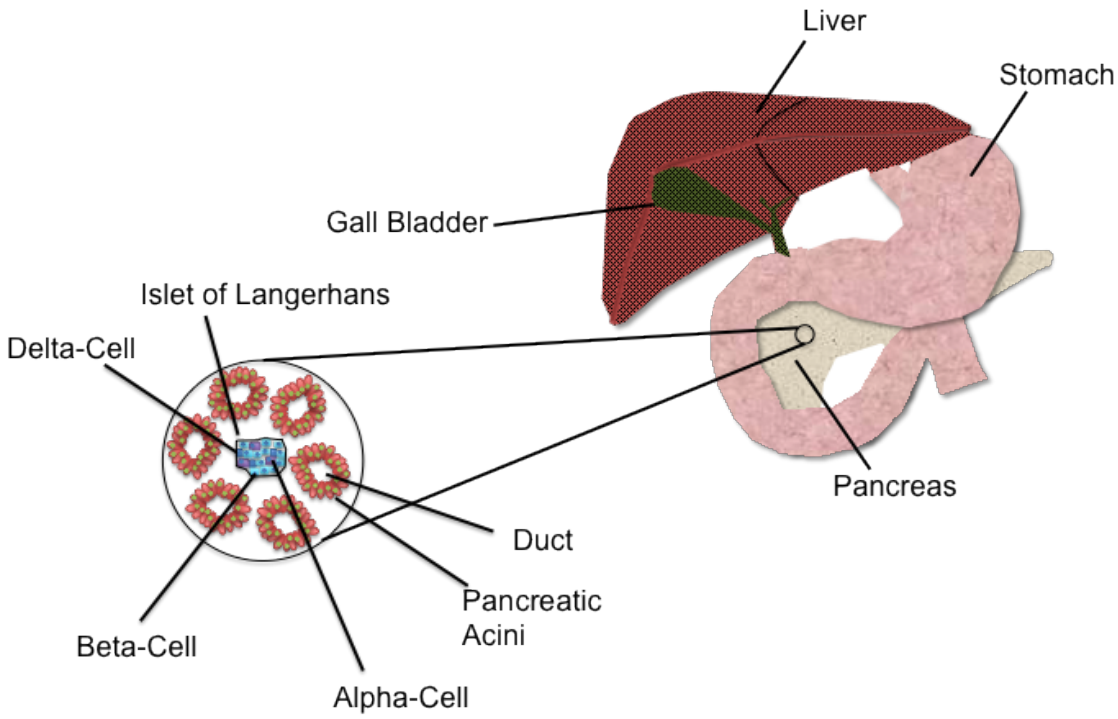

Figure 5-Anatomy of the Pancreas

Both ESC's and iPS cells have been shown to develop into insulin producing cells. Zhang *et al.* reports high effectiveness in differentiating ESC's and iPS cells via a step wise differentiation protocol in which the cells are directed to form definitive endoderm, next pancreatic specialization was induced, these progenitors cells were then expanded and induced to mature. Results showed that these cells express genes characteristic of islet cells, and insulin release was two times greater in the presence of glucose added media compared with basal media, which is comparable with the function of human adult mature islets. [26]

Islet cells ability to secrete insulin in the presence of glucose is highly-dependent on cell-cell contact in the islet. However, when ESC's are used to produce islets, larger islets can form and develop a necrotic core, and ultimately the transplantation fails, indicating the need to be able to control size of aggregate growth. Van hoof *et al.* cultured hESC's and progressively differentiated following a protocol similar to the one above, while growing the aggregates on 120 um diameter laminin coated circular patches. These cells initially grew as adherent cells but after treatment with keratinocyte growth factor formed homogenous suspended spheres averaging 100 um in diameter, which the authors report is the optimal size for these cell clusters. These cell clusters showed expression of markers positive for pancreatic endoderm.

[27]

A recent study identified a population of MSC's found in the human pancreatic islets. Differentiation of these cells was accomplished via differentiating media. The islet derived MSC's differentiated into Islet-like cells that expressed PDX1, a transcription factor associated with pancreatic development and beta-cell maturation, insulin, C peptide and Glut-2; however the control bone marrow derived MSC's only expressed Glut-2 and insulin. Insulin was also detected in the culture medium following glucose stimulation at a three fold higher rate in the islet derived MSC's than those derived from BM-MSC's. This later finding confirmed that the cells were responsive to blood glucose levels in the environment. [28]

Osteocytes/Chondrocyte Differentiation of Stem Cells

Bone possesses the natural ability to heal itself; however, if the defect in the bone is larger than a critical size; regeneration of bone tissue does not occur, and rather scarring occurs and ultimately leads to nonunion. Many approaches have been attempted that used stem cells to regenerate damaged bone tissue, the large majority of these studies employ the use of mesenchymal stem cells seeded on various scaffolds. Of these studies a large proportion also choose scaffolds will slowly degrade to allow for complete engraftment of the reformed bone. As one of the earliest studied applications of tissue engineering, there are vast amounts of trials that study the use of mesenchymal stem cells to produce bone-like tissue engineered constructs. The major property of interest is their response to the mechanical compressive forces experienced in the natural environment. Naito *et al.* show that MSC's that are cultured in hydrogels secrete larger volumes of osteocalcin, and show greater calcium deposition, which would correspondingly increase physiological function of the transplant. [29]

It should also be noted in this section, that pretreatment of MSC's using bioreactors display significantly greater mechanical properties when compared with those grown under static culture conditions. Zhang *et al.* studied the effects of several bioreactors for use in tissue engineered bone grafts. Their results show that biaxial rotating bioreactors were the ideal choice for bone tissue engineered grafts since they allow for rapid proliferation and differentiation of MSC's to osteogenic lineages as well the ability to promote homogeneous seeding of a scaffold. [30]

Early studies of mesenchymal stem cells also showed the potential of these cells to differentiate into chondrocytes. Early studies, like those conducted in osteogenic differentiation, applied the use of scaffolds for application. Edler *et al.* used bone-marrow derived MSC's, and when grown in chondrogenic media showed generation of functional hyaline-like cartilage. [31] To study the effect of transplantation on large-animal models, Jung *et al.* transplanted autologous MSC's with a collagen membrane. The grafts were then analyzed for glycosaminoglycan (GAG) content, and Collagen type II content, both of which comprise a large fraction of native cartilage. Results showed the membranes treated with MSC's still covered relatively the same size defect but the overall GAG and collagen type II content was increased in the membranes treated with MSC's. This study shows that MSC transplantation is a viable model in large-animals and may provide relief for many people with cartilage damage. [32]

Conclusion

The use of stem cell therapy in tissue engineering applications is clearly an up and coming field, providing researchers and businesses with excitement and patients with renewed hope. One important question is the type of stem cells and the source of stem cells to be used in each proposed tissue engineering therapy. Stem cells can be isolated from almost anywhere in the body, with new adult stem cell niches being found every year. Embryonic stem cells are an attractive option due to their pluripotency, but ethical concerns and regulations pose hurdles to tapping into their vast therapeutic potential. Lastly, iPSCs are the new area of research, and combine the potency of embryonic stem cells, but could be generated from any autologous cell in the future, eliminating the associated ethical concerns.

While it may take several decades to fully harness their power, the hype surrounding the use of stem cells for tissue engineering is certainly justified. There is a potential use of stem cells in almost any disorder or disease afflicting any tissue in the body. Stem cell therapies are being developed for patients suffering from heart attacks, Alzheimer's disease, Parkinson's disease, diabetes, osteoarthritis, and many other disorders. Many of these stem cell applications provide curative options to millions of people. To achieve this revolution in medicine through the use of stem cells, much more needs to be understood and characterized about these cells, and the tumorigenicity and associated immunogenicity need to be reduced. If these and other challenges are adequately addressed stem cell therapies have the potential to change the face of the world.

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