# Stem Cells and Tissue Regeneration

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The Superman actor Christopher Reeve was a symbol of strength, not only for his famous role in the movie but more so for his courage in fighting a tragic spinal cord injury. He fought courageously for 9 years, from the moment that he was thrown from his horse in 1995 until he died in 2004 at the age of 52. Spinal cord injuries can occur not only from trauma, but also from many other causes, e.g., neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS). Spinal cord injuries cause myelopathy or damage to nerve roots or myelinated fiber tracts that carry signals to and from the brain. Traumatic injury can also damage the gray matter in the central part of the cord, causing segmental losses of interneurons and motor neurons. Disappointingly, treatment options for acute, traumatic non-penetrating spinal cord injuries are still limited to the administration of anti-inflammatory agents or cold saline shortly after injury. Their effects are barely empirical and even disputable. The damaged nerves are barely repaired or regenerated due to scarring. Is there any way that they can be replaced with new, healthy neurons? The answer is yes given the discovery of neural stem cells (NSCs) and their regenerating power.

A stem cell is an undifferentiated cell that can continue dividing indefinitely, producing daughter cells, and has the capacity to differentiate into specific cell types. Stem cells are present almost in every organ in the body, and their ability to self-renew and differentiate provides the basis for tissue regeneration, permitting the body to naturally maintain homeostasis by replacing aged, damaged, and dysfunctional cells with new, healthy cells in the body. Stem cells can be classified based on their source (Figure 3.1). For example,
Embryonic stem (ES) cells are derived from early-stage embryos, and adult stem cells are tissue specific and can be isolated from almost any organ or tissue in the body. For instance, the brain is a source for NSCs, which can differentiate into many kinds of neural cells, including neurons, glial cells, and astrocytes. On the other hand, the bone marrow (BM) is a source for hematopoietic stem cells (HSCs), which can differentiate into all progeny of blood cells. Alternatively, stem cells can be categorized into totipotent, pluripotent, and multipotent stem cells, based on their differentiation ability. ES cells are pluripotent and can differentiate into any cell type in the body, whereas adult stem cells are multipotent and can usually differentiate into limited cell types for the organ or tissue from which they were isolated. In 2006, an ES-like stem cell type called induced pluripotent stem (iPS) cells was derived via the reprogramming of somatic cells with several defined factors identified.
from ES cells (Takahashi and Yamanaka 2006). Thus far, an increasing number of stem cell types have been identified and characterized (Martins-Taylor and Xu 2009). It is impossible to discuss all the stem cell types in one chapter. Therefore, we are going to use the neural (an ectodermal derivative) lineage, as well as hematopoietic, and mesenchymal (both mesodermal derivatives) lineages, as examples to introduce how scientists and physicians have been exploring the possibility of using stem cells to differentiate into or derive these lineages to treat neurological, hematopoietic, and connective tissue diseases.

3.2 ADULT NEURAL STEM CELLS

3.2.1 Location of NSCs in the Brain

In the 1960s, Drs. Joseph Altman and Gopal Das reported continuous stem cell activity in the hippocampus of the adult brain (Altman and Das 1965). As the first observation of ongoing stem cell activity in the adult brain, this finding refuted the dogma in the early twentieth century that no neurons could conceivably be generated in adulthood (Gross 2000). Subsequently, stem cell activity has also been observed in other brain regions (Altman 1969; Reynolds and Weiss 1992; Weiss et al. 1996). As shown in Figure 3.2, adult NSCs are located primarily in two regions: the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus (Gage 2000). What is the potential for differentiation in these adult NSCs? Will these stem cells be activated after injury or under pathological conditions? Can they be used in therapies for brain disorders? These questions will be discussed in the following sections.

3.2.2 Differentiation Potential of Adult NSCs

NSCs can renew themselves and differentiate into multiple cell types of the neural lineage (Ming and Song 2005). Adult NSCs in the SVZ and SGZ are positive for GFAP and CD133. Under physiological conditions, NSCs in the SVZ develop into intermediate progenitors,
which then migrate to the olfactory bulb and differentiate into GABAergic interneurons (Lois et al. 1996; Curtis et al. 2007). In contrast, SGZ progenitors (also positive for Sox2), as shown in fate-tracing studies, give rise to neurons and astrocytes (Suh et al. 2007). Although NSCs in both areas are multipotent in Petri dish cultures, it is unclear whether a single NSC can give rise to multiple neural cell types in the brain under physiological conditions.

Adult NSCs can be isolated from the adult brain in animals (Morshead et al. 1994; Bonaguidi et al. 2008), brain biopsy and postmortem tissues in humans (Palmer et al. 2001), and expanded in vitro as free-floating clusters (neurospheres) in the presence of mitogens, such as bFGF and EGF. These cells have the two hallmarks of stem cells, in that they can self-renew and have the capacity to differentiate into multiple cell types in the neural lineage, such as neurons, astrocytes, and oligodendrocytes. At the clonal level, adult NSCs can form neurospheres and exhibit multipotency (Palmer et al. 1997). Adult NSCs give rise primarily to GABA interneurons and glutamatergic neurons but not other neuronal subtypes, such as spinal motor neurons (large projection neurons that form during early development) (Temple 2001). In addition to the SVZ and SGZ, neurospheres can also form after isolating cells from other regions of the brain (striatum and cortex) and spinal cord (Palmer et al. 1995; Weiss et al. 1996). Under physiological conditions, these areas do not undergo neurogenesis in vivo. However, after injury or under pathological conditions, endogenous stem cell activity is also observed in other areas of the brain, which will be discussed later. What is the niche for NSCs and how are the activation and differentiation of NSCs regulated? Insights into these questions will provide critical information for the application of NSCs in neurological diseases.

3.2.3 Therapeutic Applications of Adult NSCs in Neurological Disorders

The first step toward using adult NSCs for neurological diseases is to activate or recruit adult NSCs to replace the cells that have been lost. To a limited extent, the brain can replace and repair neurons that have been lost after injury (Emsley et al. 2005). In 2002, several groups examined the neurogenesis and generation of neuron after focal ischemia in rodents (Nakatomi et al. 2002; Parent et al. 2002; Kokaia and Lindvall 2003). Ischemia induces significant increases in the proliferation of the SGZ and SVZ. New neurons, such as hippocampal pyramidal neurons and striatal projection neurons that are lost in ischemia, were also generated in the ischemic region (Parent et al. 2002). One study also showed that infusing growth factors into the ventricle enhanced the generation of new neurons (Nakatomi et al. 2002). These newly generated neurons were electrophysiologically active and effected improvements in cognitive function after stroke.

Increases in SGZ neurogenesis have been observed after temporal lobe epilepsy, which usually leads to hippocampal cell damage; some NSCs in the SGZ migrated into the hilus and differentiated into mature granule neurons (Parent et al. 1997). It has also been shown that seizures promote synapse integration in nascent neurons (Overstreet-Wadiche et al. 2006). But, newly formed ectopic granule neurons might cause recurring seizures (Parent et al. 2006), suggesting that the proper synaptic connections and circuitry are important to experience functional improvements in seizures. Whether and how adult NSCs can give rise to certain neuronal subtypes and integrate into the circuitry in vivo remain to
be elucidated and will be important for the development of endogenous cell replacement therapies for neurological diseases.

Another strategy for using NSCs in neurological diseases is the transplantation of NSCs from adult or, more likely, fetal brain (Lindvall and Kokaia 2006; Einstein and Ben-Hur 2008; Kim and de Vellis 2009). After transplanting the neurospheres that are formed from NSCs into the brain, these cells can also generate neuron and glial cells in vivo (Gage et al. 1995; Suhonen et al. 1996). NSCs have been transplanted into experimental models of Huntington disease (HD) (Kordower et al. 1997; Lee et al. 2005), ALS (Xu et al. 2006; Hwang et al. 2009), Parkinson disease (PD) (Love et al. 2005; Emborg et al. 2008), and stroke (Kim et al. 2008), etc. The survival of grafted cells and functional improvements has been reported.

3.2.4 Success and Limitations of NSC-Based Therapy

Some clinical trials are aimed at stimulating the differentiation of adult NSCs. For example, treatment with G-CSF and cistanche total glycosides is being assessed in ALS patients. Since the availability of adult NSCs from humans is limited, clinical trials that use transplanted adult NSCs are relatively difficult. In 2009, the Rajavithi Neuronal Adult Stem Cells Project (RNASC) in Thailand initiated a phase II clinical trial (clinicaltrials.gov). It is aimed at identifying unlimited human neuronal progenitor stem cells from the human brain from patient biopsies from either elective or emergency surgeries and generating oligodendrocytes progenitors, as well as other neuronal subtypes in the next phases, for treatment of Alzheimer’s disease, multiple sclerosis (MS), and PD. Another strategy is to recruit or activate endogenous NSCs. However, success via this approach largely depends on more in-depth understanding of the mechanisms that regulate the activation and differentiation of NSCs and the functional integration of NSC-derived neurons. Due to the difficulty of obtaining NSCs from humans, scientists have been exploring other sources to procure human neural cells, as well as other therapeutic cells, in large quantity. Since ES cells can be in vitro differentiated into various cell types and be expanded without limitation, they are ideal for this purpose.

In February 2010, the United Kingdom Gene Therapy Advisory Committee announced that it had approved ReNeuron’s phase I clinical trial for testing a stem cell–derived therapy on stroke patients (http://www.reneuron.com/news_events/news/document_178_237.php). This follows the approval from the UK Medicines and Healthcare Products Regulatory Agency. In developed countries, stroke is the third largest cause of death and the single largest cause of adult disability. The vast majority of strokes are ischemic, which is caused by a blockage of blood flow in the brain. As a result of the damage to the brain, stroke survivors are often left with permanent disabilities. Treating patients with anti-clotting agents within hours of the stroke is currently the major treatment for ischemic stroke patients. Unfortunately, most patients do not get to the hospital in time for the treatment. The late-phase treatment of ischemic stroke victims is rehabilitation measures. In this phase I clinical trial, safety and efficacy were assessed about injecting NSCs, derived from human fetal cortex at 14 week gestation, into disabled ischemic stroke patients between 6 and 24 months after their stroke. Preclinical testing in animal models of ischemic stroke...
has been promising in that many of the functional deficits associated with disabilities caused by strokes are reversed (reviewed in Gutierrez et al. 2009).

3.3 EMBRYONIC STEM CELLS

3.3.1 Early Embryogenesis

ES cells are derived from early-stage embryos. To understand the nature of the source cells, here we will briefly introduce the early embryogenesis (Figure 3.1). Upon fertilization, a mammalian embryo undergoes sequential cell divisions or cleavages, going through the 1-, 2-, 4-, and 8-cell stages. During these early stages, the cells, also called blastomeres, within the embryo are loosely stuck together. When the embryo undergoes cell division from the 8-cell to the 16-cell stage, the cells tightly compact to form a ball of cells called a morula. There are tight junctions between the cells that seal the interior of the morula from the external medium. As a result, an internal cavity forms, converting the morula into a blastocyst. The outer layer of cells of the blastocyst is called trophectoderm, which will give rise to extraembryonic tissue, including the amniotic sac and placenta. The amniotic sac and the placenta enclose and protect the embryo proper. Moreover, they supply the embryo proper with the metabolites required during development from the mother. These tissues are referred to as extraembryonic tissues, since they do not form any part of the adult and are discarded at birth. The cavity of the blastocyst contains an inner clump of cells called the inner cell mass (ICM) that is located to one side. The cells within the ICM differentiate to form the embryo proper and other extraembryonic tissues, such as the yolk sac. The individual cells within the ICM are pluripotent, meaning that they can give rise to any cells within the adult body, including germ cells.

The cells of the ICM differentiate to form the three primary, embryonic germ layers: ectoderm, mesoderm, and endoderm. The transformation from the sphere of cells of the ICM into a structure with a gut is called gastrulation. During gastrulation, the germ layers are positioned such that layers can develop into bodily systems. The ectoderm is the outermost layer and differentiates first, forming the precursor of the epidermis (external ectoderm) and the nervous system (neuroectoderm). The mesoderm forms the middle layer and can differentiate into a number of tissues, including bone, muscle, connective tissue, and the middle layer of the skin. The endoderm is the innermost layer and is the precursor to the gut and its appendages, including the liver and lung. In one form or other, this is the universal feature of animal development.

3.3.2 Derivation and Characterization of Mouse ES Cells

ES cells were first derived by Martin Evans and Gail Martin laboratories from mouse embryos in 1981 by isolating and culturing the ICM of mouse blastocysts in serum-containing medium on mitotically inactivated mouse embryonic fibroblasts (MEFs) as a feeder layer (Evans and Kaufman 1981; Martin 1981). Mouse ES cells have normal karyotype, although they can become unstable in extended culture. They highly express transcription factors Oct3/4, Nanog, and Sox2 among many other pluripotency-sustaining factors. Mouse ES cells also express cell surface antigen SSEA-1 and alkaline phosphatase.
It has been known that two cytokines, leukemia inhibitory factor (LIF) (Yoshida et al. 1994) and bone morphogenetic proteins (BMP) (Ying et al. 2003), in replacement of serum, can maintain the culture of mouse ES cells and prevent them from differentiation. Inhibitors of two signaling kinases ERK and GSK-3 (the 2i system), which presumably serve the same target as for LIF and BMP, can also maintain their undifferentiated expansion (Ying et al. 2008).

Mouse ES cells can be differentiated in vitro into cell types that represent all the three embryonic germ layers, through directed differentiation or formation of embryoid bodies (EBs). EBs are formed by dissociating the ES cell colonies into smaller clumps and culturing them using methods that prevent their attachment (i.e., in hanging drops or in low-attachment tissue culture flasks). ES cells start to differentiate with the EB to a limited extent by recapitulating embryonic development, which gives rise to precursors that can differentiate into cell types from the three germ layers, as well as extraembryonic lineages. ES cells can also form teratomas when injected into immunodeficient mice. Teratomas are encapsulated tumors that contain tissues from the three germ layers. Furthermore, they are capable of producing chimeras. Mouse ES cells can be injected into an early-stage embryo from a different strain of mice and develop into an adult chimeric mouse. The adult chimeric mouse is composed of cells from both strains. Coat color is often used as a marker for chimerism. Mouse ES cells are also capable of producing chimeras through germline transmission (i.e., transmission via gametes to offspring), which is the ultimate test of pluripotency. The derivation and characterization of mouse ES cells have set the basic methods and standards for the subsequent derivation of ES cells from other species, including human.

3.3.3 Derivation and Characterization of Human ES Cells

Seventeen years after mouse ES cells were first derived, James Thomson’s laboratory derived ES cells from human blastocysts in 1998 (Thomson et al. 1998), based on their previous success in derivation of monkey ES cells from rhesus and Marmoset (Thomson et al. 1995, 1996). It is undoubtedly a breakthrough progress for this important research from animals to humans. Advances in the culture conditions for human embryos in in vitro fertilization clinics also contributed to the derivation of human ES cells (Gardner et al. 1998). Similar to other ES cells, human ES cells are capable of long-term self-renewal, maintain a normal karyotype, and can differentiate into cell types that represent the three germ layers via EB or teratoma formation. Like monkey ES cells, human ES cells express the surface markers SSEA-3, SSEA-4, TRA1-60, and TRA1-81.

The growth factors required for human ES cell self-renewal are distinct from those required for mouse ES cells. Human ES cells do not require LIF for the maintenance of self-renewal (Humphrey et al. 2004; Sumi et al. 2004). Moreover, BMPs induce human ES cell differentiation into trophoderm and primitive endoderm (Xu et al. 2002; Pera et al. 2004). We (Xu et al. 2005, 2008) and others (reviewed in Okita and Yamanaka 2006; Watabe and Miyazono 2009) have shown that FGF signaling, as well as TGF-β/activin/nodal signaling, are required for human ES cell self-renewal. Ligands of these two signaling pathways can sustain long-term culture of human ES cells although, like mouse ES cells,
human ES cells can also be derived and maintained on MEF as feeders and in medium conditioned by the feeders. These feeder cells have been shown to provide multiple ligands to activate these essential pathways and inhibitors to block the differentiation pathways.

3.3.4 Potential Applications of ES Cells to Treat Degenerative Diseases

Thus far, ES cells have been also derived from avian, monkey, rat, rabbit, and dog, but mouse ES cells are mostly used for modeling and treating human diseases both in vitro and in vivo (Martins-Taylor and Xu 2009). Thus, the derivation of ES cells from these species has created important resources for studying these diseases and for pharmaceutical research. These ES cell lines may be used for developing and testing potential therapies for treatment of human diseases. However, the therapeutic promises of ES cell progeny depend on our knowledge and our ability to drive ES cells differentiation into the particular cell types desired. Great efforts are being made to understand and recapitulate the niches that control differentiation into specific lineages. ES cells cannot be directly transplanted into patients due to their pluripotency that can cause teratomas in the host.

Progress has been made to differentiate ES cells into lineage-specific precursors that may be used in regenerative medicine applications. In particular, differentiation of ES cells into neuronal lineages has been extensively studied and thus, reproducible generation of different subtypes of neurons is possible. Degeneration of certain types of neurons is implied in many neurodegenerative disorders, such as PD, HD, and ALS. In addition to neurons, astrocytes and oligodendrocytes are two important cell types in the central nervous system. Congenital dysmyelinating and acquired demyelinating diseases are caused by degeneration of oligodendrocytes, the sole source of myelin in the central nervous system. Currently, there is no effective treatment or prevention for these diseases, partially due to the lack of a system to study these diseases and screen drugs. Thus, generation of neuronal progenitors from ES cells holds promise for studying human neurogenesis, developing assays for pharmacological and toxicological studies, and developing potential cell therapy applications.

3.3.5 Differentiation of Functional Neural Cells from ES Cells

During development, neural induction is initiated when BMP signaling is inhibited, as we (Xu et al. 1995) and others (Sasai et al. 1995; Dale and Jones 1999) first demonstrated in Xenopus embryo, or when FGF signaling is activated (Wilson and Edlund 2001). Administration of a BMP antagonist (Noggin), basic FGF, and other factors spurs efficient neural differentiation from mouse and human ES cells. The most commonly used methods of neural induction involve adherent cultures (Chambers et al. 2009), EB formation (Zhang et al. 2001; Li and Zhang 2006), and coculture with stromal cells (Tabar et al. 2005; Pomp et al. 2008). Because the latter promotes the development of neural cells with a midbrain/hindbrain identity, this technique has been used widely to generate midbrain neurons.

Of the approaches that entail EB formation (or the aggregation of ES cells), a chemically defined system established by the Su-Chun Zhang laboratory (Zhang et al. 2001; Li and Zhang 2006) has been widely used. Briefly, ES cell colonies are detached from their feeder layers or matrices to initiate differentiation. The ES cell aggregates are kept in suspension in ES cell medium for 4 days and in a neural induction medium for the next 2 days, which
mimics gastrulation and induces the formation of ectodermal germ layers. These ES cell aggregates adhere to the culture surface and form columnar neuroectodermal cells that organize into neural tube-like rosettes after 14 days of differentiation. These neuroectodermal cells can be isolated through enzymatic treatment and adhesion. This approach not only generates neuroectodermal cells at high efficiency, but it also recapitulates early embryonic development with regard to timing and morphology.

Neuroectodermal cells further pattern into regional progenitors along the dorsal–ventral and rostral–caudal axes (Briscoe and Ericson 2001), which subsequently develop into distinct neuronal subtypes that have different biochemical functions including secretion of neurotransmitters. Because the mechanism by which neurons express certain neurotransmitters is unknown, the specification of neuronal subtypes from ES cells is based primarily on their positional identities (Zhang 2006). Several neuronal subtypes, including spinal motor neurons (Li et al. 2005; Singh Roy et al. 2005; Lee et al. 2007) and midbrain dopaminergic neurons, have been successfully specified from ES cells with retinoic acid (RA)/sonic hedgehog (SHH) and FGF8/SHH, respectively. RA and FGF8 promote the specification of cells in the spinal cord and midbrain, respectively. SHH, an important ventralizing factor, can be used to specify spinal motor neurons and midbrain dopaminergic in culture.

The identities of such neurons have been confirmed based on the expression of various markers of neuronal position and neurotransmitters. Moreover, these neurons are electrophysiologically active, release dopamine upon stimulation (for dopaminergic neurons) (Perrier et al. 2004; Yan et al. 2005), or make functional connections with target muscle cells (for motor neurons) (Li et al. 2005; Lee et al. 2007). Generation of other cell types, such as oligodendrocytes (Nistor et al. 2005; Hu et al. 2009), retinal photoreceptor (Osakada et al. 2008), GABAergic neurons (Chatzi et al. 2009), and forebrain glutamatergic neurons (Li et al. 2009b), has also been reported.

3.3.6 ES Cell–Derived Neuronal Cells for Treatment of Neurological Diseases in Animal Models

To explore the potential application of ES cell–derived neurons in regenerative medicine, these neurons have been transplanted into animal models to evaluate their survival and function in the hosts (Zhang et al. 2008). Most of these studies have focused on several common neurodegenerative diseases, e.g., PD, ALS, HD, and spinal cord injury.

3.3.6.1 Parkinson Disease

PD is characterized by selective loss of dopaminergic neurons in the substantial nigra, resulting in muscle rigidity, tremor, and a slowdown or loss of movement. Due to the highly selective cell loss in a restricted location, PD is an ideal target for cell replacement therapy. Ron Mckay laboratory transplanted normal mouse ES cells and Nurr1-overexpressing mouse ES cells into 6-OHDA-lesioned animals (a PD model), demonstrating that Nurr1-overexpressing ES cells differentiate into TH-positive dopaminergic neurons after transplantation, effecting functional improvements, based on behavioral changes in these animals. Subsequently, monkey ES cells were induced to assume the dopaminergic
phenotype and were transplanted into MPTP-lesioned monkeys, where differentiation of dopaminergic neurons and functional improvement were observed after transplantation.

Although human ES cells can also differentiate into dopaminergic phenotype in vitro, the rates of survival and differentiation of TH+ dopaminergic neurons in vivo are low. Goldman laboratory cotransplanted human ES cells with midbrain astrocytes, which improved the survival and differentiation of dopaminergic neurons from human ES cells (Roy et al. 2006). Although the recipients exhibited a decrease in rotation on stimulation, some animals rotated in the opposite direction, which might have been caused by the over-release of dopamine by the transplanted cells.

3.3.6.2 Amyotrophic Lateral Sclerosis

Motor neurons are large projection neurons that control all muscle movements. The degeneration of motor neurons is implied in certain devastating disorders, such as ALS and spinal muscular atrophy (SMA). Mouse and human ES cells have been successfully differentiated into spinal motor neurons that can make synaptic connections with muscle cells in vitro (Wichterle et al. 2002; Li et al. 2005; Lee et al. 2007). Because the pathway toward motor neuron axons and the functional connection between muscle cells and motor neurons are controlled precisely during embryonic development, it has been difficult for grafted motor neurons to grow out of the boundary between central and peripheral nervous systems (CNS–PNS) and home to the correct targets (the distance can be 1 m in adults compared with several millimeters in developing embryos).

After transplantation into chick embryos at the stage in which endogenous motor neurons are developed, mouse ES cell–derived motor neurons survive and grow axons out of the spinal cord that make functional connections with muscle cells (Wichterle et al. 2002). Upon combined treatment with an inhibitor of CNS–PNS boundary formation and a target-derived factor, mouse ES cell–derived motor neurons sprout several axons (Harper et al. 2004). A subsequent study by the same group improved this method and used an additional inhibitor to overcome the myelin-mediated repulsion during transplantation, which resulted in the recovery from paralysis in adult rats that were grafted with mouse stem cells (Deshpande et al. 2006).

Although motor neurons are specifically lost in ALS, the loss of motor neurons in ALS is not cell autonomous, and glial cells (both astrocytes and microglial cells) regulate the development and acceleration of the pathogenesis in these patients. Within the affected glial cells, transplanted motor neurons may not survive. Recently, a group reported the survival and functional improvement of ES cell–derived glial cells after transplantation (Lepore et al. 2008). Considering the difficulties in the outgrowth and homing of motor neuron axons, transplantation of glial cells might be a more effective and practical method to protect the remaining motor neurons in patients (Nayak et al. 2006).

3.3.6.3 Huntington Disease

HD is an inherited, autosomal dominant, neuropsychiatric disease that is caused by expansion of a polyglutamine tract in the Huntingtin protein, which results in significant neuronal loss and atrophy in the brain. The striatal projection neuron (spiny medial
GABAergic neuron) is the most important cell type that degenerates in HD. A recent study differentiated human ES cells into neurons that had striatal GABAergic neuronal phenotypes and transplanted them into the quinolinic acid–lesioned nude rats whose striatal neurons were injured and depleted, mimicking HD pathology (Aubry et al. 2008). Striatal progenitors generated many NESTIN+ early neural progenitors 4–6 weeks after transplantation. A small population of cells differentiated further into DARPP32+ striatal GABAergic neurons in rat brain 3–5 months after transplantation. However, the functional and behavioral changes in these rats were not examined.

3.3.7 Transplantation of ES Cell–Derived Oligodendrocyte Progenitors
ES cell–based therapy has also been applied to animal models of CNS injuries, such as spinal cord injury (Coutts and Keirstead 2008). Human ES cell–derived oligodendrocyte progenitors were transplanted into injured adult rat spinal cords, which resulted in improved locomotor ability and movement recovery (Keirstead et al. 2005). Oligodendrocytes supply myelin in the CNS, and enhanced remyelination was observed in these animals after transplantation. In addition to spinal cord injury, ES cell–derived oligodendrocytes also represent a source of myelin in myelin-related diseases, such as congenital hypomyelination and MS (Goldman 2005; Windrem et al. 2008).

Based on the success of the transplantation studies in animal models with spinal cord injury, the first clinical trial of human ES cells, which will be conducted by Geron Corporation, was approved by the U.S. Food and Drug Administration in January 2009 (http://www.geron.com/media/pressview.aspx?id=1148). In this phase I clinical trial, Geron Corporation will implant human ES cell–derived oligodendrocyte progenitors into 8–10 paraplegic patients who can use their arms but cannot walk. These patients have no other alternative methods available to regain the functions lost by their injuries. Patients with injuries in the middle of the spinal cord between the third and tenth vertebrae will receive injections at the site of the injury. Moreover, these patients must have sustained their injuries with 7–14 days to be included in this groundbreaking clinical trial. The primary goal of this study, according to Geron, is to determine whether injecting ES cell progeny into humans is safe, and also to monitor sensation and movement in the legs of the patients.

3.3.8 Challenges and Perspectives
Although several neuronal subtypes have been successfully specified from ES cells and functional improvements have been observed after transplanting these cells into animal disease models, many challenges still remain in this field. The most important issue is the safety of ES cell transplantation. Because ES cells can differentiate into any cell type, they can form teratomas after transplantation. Although most transplantation studies have used differentiated cells, proliferation of grafted cells has been observed several months after transplantation in many studies. To circumvent this potential, one can purify the transplanted cells or eliminate pluripotent stem cells by using flow cytometry–based cell sorting or drug selection.

In addition, there are other problems that need to be solved, such as how to improve and achieve the survival and integration of transplanted cells into the host environment and how to encourage the outgrowth and homing of motor neuron axons after transplantation.
Studies in these areas will provide important insights and facilitate the success of stem cell–based therapies for neurological diseases. Another challenge of ES cell–based therapy is immunorejection, because it is impossible to develop personalized ES cells. The use of early-stage human embryos is ethically disputable, although they are donated to research by infertility patients after treatment is completed. Recent advances in generating iPS cells by reprogramming somatic cells may provide solutions to overcome these hurdles.

3.4 INDUCED PLURIPOTENT STEM CELLS

Following the first derivation of human ES cell lines, scientists began seeking alternative approaches to deriving ES-like cells from cells that carry the same genotype as a patient, without using embryos, in order to circumvent the ethical concerns surrounding the disputable use of donated early human embryos for the derivation of ES cells. This was elegantly achieved by Yamanaka and Takahashi in 2006, who discovered that four essential transcription factors that are highly expressed in ES cells can be used to reprogram somatic cells into an embryonic-like state. This led to the creation of another new pluripotent stem cell type, known as iPS cells (Takahashi and Yamanaka 2006). This new technology has been quickly recapitulated in human (Takahashi et al. 2007; Yu et al. 2007) and many other species including monkey (Liu et al. 2008; Wu et al. 2010), pig (Esteban et al. 2009; Ezashi et al. 2009; Wu et al. 2009), rat (Li et al. 2009a; Liao et al. 2009), and canine (Shimada et al. 2010).

3.4.1 Derivation of iPS Cells

The first differentiated source cells for iPS cell derivation were MEFs and mouse adult tail fibroblasts (Takahashi and Yamanaka 2006). Retroviral transduction was used to express the reprogramming factors Oct4, Sox2, Klf4, and c-Myc. To sustain their pluripotency, mitotically inactivated MEFs were used as feeders in the derivation and maintenance of mouse iPS cells. The iPS cells exhibit similar features as mouse ES cells, including staining positive for alkaline phosphatase and SSEA-1. Moreover, they can be differentiated in vitro into cell types that represent all the three embryonic germ layers, through directed differentiation or formation of EBs, and form teratomas when injected into SCID mice. Furthermore, they are capable of producing chimeras with germline transmission (Maherali et al. 2007; Okita et al. 2007; Wernig et al. 2007).

Two independent groups using partially different combinations of reprogramming factors derived the first human iPS cell lines. Thomson group used OCT4, SOX2, NANOG, and LIN28 via lentiviral transduction (Yu et al. 2007), whereas Yamanaka group used the reprogramming factors and retroviral transduction (Takahashi et al. 2007), the same factors that were used for the mouse iPS cells (Takahashi and Yamanaka 2006). Similar to human ES cells, human iPS cells are derived on MEF feeders and cultured under the same conditions. Moreover, human iPS cells express the same cell surface markers as human ES cells, which include SSEA-3, SSEA-4, TRA1-60, and TRA1-81. Cell types from all three germ layers can be differentiated in vitro from human iPS cells. Furthermore, when injected into immunodeficient mice, they form teratomas. Thus, iPS cells appear to have the same developmental potency as ES cells.
3.4.2 Methodological Improvement

Since these groundbreaking reports, iPS cells have been generated from various tissues such as keratinocytes, B lymphocytes, BM cells, liver cells, NSCs, and meningeocytes (reviewed in Feng et al. 2009). One or more viral factors can be replaced and reprogramming efficiency can be enhanced through the use of pharmaceutical inhibitors that specifically target epigenetic modifiers or pluripotency regulators (reviewed in Feng et al. 2009). Virus-free methods have been developed for the derivation of iPS cells. To prevent transgene integration in the genome, adenoviral transduction (Stadtfeld et al. 2008), transient transfection (Okita et al. 2008), and the piggyBac (PB) transposon gene-delivery systems (Kaji et al. 2009; Woltjen et al. 2009; Yusa et al. 2009) have been used for reprogramming mouse cells. Moreover, iPS cells can be generated from mouse and human differentiated cells via the transduction of recombinant OCT4, SOX2, KLF4, and c-MYC proteins, free of any DNA delivery (Bru et al. 2008; Zhou et al. 2009). Furthermore, human iPS cells can be generated using serial RNA transfections with mRNA that was synthesized in vitro cloned from the cDNA of OCT4, SOX2, KLF4, and c-MYC (Yakubov et al. 2010). These are remarkable progresses for preserving the integrity of the host genome, but the cost of the derivation appears very high while the efficiency being extremely low.

iPS cells have been recently derived and maintained in the completely defined medium mTeSR1 (Stem Cell Technologies, Vancouver, Canada) (Ludwig et al. 2006) from human adipose stem cells with the Yamanaka factors via lentiviral transduction (Sun et al. 2009). Furthermore, iPS cells have been derived from rat, monkey, porcine, and canine, creating important resources for studying human disease and pharmaceutical research. These animal models are used to study numerous human diseases, especially in the development and testing of potential therapies.

3.4.3 Potential Clinical Applications of iPS Cells

There is great potential for the use of iPS cells in therapeutic applications for regenerative medicine. Proof-of-principle experiments have been done using mouse iPS cells in a humanized sickle cell anemia mouse (Hanna et al. 2007). Sickle cell anemia or sickle cell disease is a painful genetic disorder that affects the shape of red blood cells, resulting in a loss of elasticity. The red blood cells change shape or sickle in low oxygen conditions. However, these cells fail to return to normal shape when normal oxygen tension is restored. These rigid red blood cells are unable to pass through narrow capillaries, resulting in blood vessel blockages and restriction in the blood supply, which can damage tissues. Moreover, the sickled red blood cells are destroyed in the spleen, causing anemia. This disease is caused by mutations in the hemoglobin gene. In these experiments, gene targeting and reprogramming were coupled to correct the mutation in the hemoglobin gene. When hematopoietic progenitors that were derived from the iPS cells were transplanted into the mouse model, the sickle cell phenotype can be rescued. Proof-of-principle experiments have also been done using iPS cell–derived dopaminergic neurons in a rat model of PD (Wernig et al. 2008). The transplanted dopaminergic neurons were able to improve behavior of the rats. These findings demonstrate the therapeutic potential of iPS cells in regenerative medicine. The progeny of patient-specific
iPS cells may also be used as a tool for diagnostic testing of disease and screening for drugs most effective for the patient.

3.4.4 Challenges and Perspectives

Although the iPS cell technology has eliminated the need for human embryos and avoided immunorejection of stem cell progeny by recipients, there are still some preexisting and new challenges. For example, the iPS cell derivation efficiency is still very low, especially for virus-free methods. The efficiency for iPS cells to differentiate into desired cell lineages for therapy appears to be even lower than that for ES cells (Choi et al. 2009; Feng et al. 2010; Hu et al. 2010; Kim et al. 2010; Kulkeaw et al. 2010). Teratoma-forming risk remains if any therapeutic cells are mixed with residual, undifferentiated pluripotent stem cells. There is also risk of oncogenesis due to the disruption of the host genome or reactivation of the reprogramming factors in iPS cell progeny, if the iPS cells were reprogrammed via viral transduction. In-depth study is necessary to understand the fate of the iPS cell progeny in vivo, for example, their survival, integration, and functionality. Finally, we must continue to develop various animal-free conditions for lineage-specific differentiation of the stem cells so that their differentiated cells are directly at clinical grade.

Earlier, we used application of stem cell–differentiated neurogenic cells to treat neurological diseases as an example. Obviously, ES or iPS cells and many other stem cell types have been studied or used for therapy of non-neurological diseases. We will introduce some of them later to broaden our knowledge of these additional aspects.

3.5 HEMATOPOIETIC STEM CELLS

HSCs are multipotent stem cells that can differentiate into all blood cell types including myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells) and lymphoid lineages (T cells, B cells, NK cells). HSCs also have plasticity to transdifferentiate into other cell types such as muscle, endothelial cells, and bone cells (Hombach-Klonisch et al. 2008). HSC transplantation is the earliest stem cell-based therapy that started in 1950s and has been widely used to treat cancers, as well as many blood and immune system disorders. Currently, human BM, mobilized peripheral blood, and umbilical cord blood (UCB) represent the major sources of transplantable HSCs (Copelan 2006). However, there are many drawbacks to the current sources of all these cells. The availability of these cells is dramatically limited due to the limited number of tissue-derived HSCs, the limited expansion of the HSCs in vitro, rare histocompatibility or match of human leukocyte antigen (HLA) types between donors and recipients, and the risk of transmission of infectious diseases. Thus, sources with robust, continuous supply of hematopoietic cells are highly desired. Human ES and iPS cells provide great promise for this purpose.

3.5.1 Differentiation of HSCs from Human ES Cells

For treatment of hematopoietic diseases, human ES cells must be first differentiated into hematopoietic cells. Differentiation methods can be categorized into two main approaches: coculture on supportive stromal cell layers and the formation of EBs. Cell lines have been
used in stromal coculture systems. One benefit of differentiating human ES cells using these stromal cell lines in coculture is the ability to control the specific elements needed to provide a microenvironment or anatomical niche that supports lineage-specific development. Specific stromal cell lines can be selected to generate a desired progenitor type. Moreover, the stromal cell lines also can be genetically engineered to enhance progenitor quality and number. For example, expression of Wnt1 in S17 cells increases hematopoietic differentiation from human ES cells (Woll et al. 2008). OP9-DL1 cell line, which expresses a Notch ligand, promotes the differentiation into lymphoid lineages (Schmitt et al. 2004). However, the involvement of animal-derived stromal cells and the variation of yield limit the use of these methods. Three-dimensional EB differentiation cultures have also been used to differentiate human ES cells into hematopoietic cells (Chadwick et al. 2003). This method yields more consistent and efficient results; however, appropriate EB sizes are critical (Ng et al. 2005).

To characterize human ES cell–derived hematopoietic cells, common markers such as CD34, CD45, c-Kit, and Lin can be used for characterization of HSCs. Many studies suggest that from human ES cell–derived HSCs have a similar profile of the markers and can reconstitute certain lineages in immunodeficient mice. However, long-term reconstitution of multilineage hematopoietic cells in mouse models has not yet succeeded for human ES cell–derived HSCs, in comparison with HSCs derived from BM or umbilical blood (Kaufman 2009).

### 3.5.2 Lineage-Specific Differentiation of Human ES Cell–Derived HSCs

Human ES cell–derived HSCs have been successfully differentiated in vitro into several mature blood cell lineages, which hold great potential for applications in regenerative medicine.

#### 3.5.2.1 Erythroid Cells

Although the current red blood cell supply is adequate and relatively safe, the need for rare blood groups and the risk of contamination by new pathogens cannot be completely eliminated. Thus, derivation of erythroid cells from human ES cells may translate into a new and safer source of red blood cells for transfusions. Recently, Advanced Cell Technology, Inc. has successfully produced large amounts of red blood cells from human ES cells, using a procedure that involves a combination of EB formation and the ectopic expression of the HoxB4 transcription factor (Lu et al. 2008). Through the amplification and maturation of hematopoietic and erythroid precursors, they were able to obtain enucleated red blood cells on a feeder layer. This is a great progress toward the application of human ES cell–derived erythroid cells in potential therapies.

#### 3.5.2.2 T, B, and Natural Killer Cells

OP9 coculture and EB formation can also be used to derive T and B cells from human ES cell–derived HSCs, using cocktails of cytokines at multiple steps within differentiation. T cells can be differentiated by coculture with the OP9-DL1 stroma cell, while B cells can be differentiated by adding FLT-3 ligand to the culture during the early lymphopoietic
progenitor stage (Cho et al. 1999). However, the engraftment of these cells in the immuno-compromised SCID mice is typically 1% or less (Wang et al. 2005; Narayan et al. 2006). In contrast, T cells derived from human BM, UCB, or fetal liver have much higher levels of engraftment in SCID mice. This indicates that T and B cells derived from these various sources may carry intrinsic differences.

The \textit{in vitro} differentiation of human ES cell–derived HSCs into NK cells is less difficult, which was first demonstrated by Dan Kaufman laboratory. Functional NK cells can be efficiently generated from human ES cells, using a two-step culture method (Woll et al. 2005). The human ES cells are first cocultured with the stromal cell line S17. The resulting human ES cell–derived HSCs are then cocultured with AFT024 cells. Interestingly, these NK cells can kill multiple cancers both \textit{in vitro} and \textit{in vivo} and the \textit{in vivo} killing is more effective for NK cells derived from human ES cells than those from UCB cells (Woll et al. 2009).

3.5.2.3 Macrophages and Dendritic Cells
Macrophage and dendritic cells have very important immune functions and are good candidates for cell therapy to treat cancer and immune diseases. Multiple groups have reported the derivation of macrophage and dendritic cells from human ES cells, mainly by using the stromal cell coculture system to first generate HSCs (Slukvin et al. 2006). Subsequently, these HSCs are induced to differentiate into macrophages upon the addition of the cytokines M-CSF and GM-CSF, or dendritic cells upon the addition of the cytokines GM-CSF and IL-4 (Slukvin et al. 2006).

3.5.3 Challenges and Perspectives
Human ES cell–derived hematopoietic progenitors and immune cells have several advantages for potential clinical application. First, unlimited numbers of HSCs can be generated from self-renewing human ES cells, in contrast to the limited source from BM and UCB. Second, the transplantation of human ES cell–derived HSCs, along with other cell lineages such as islet cells, neurons, and muscle cells derived from human ES cell lines, may help HSCs to engraft and function by reducing transplantation rejection and inducing immune tolerance (Priddle et al. 2006). Finally, the derivation of HSCs derived from human ES cells may provide opportunities to genetically engineer or modulate antigens at different stages of hematopoietic development, which may be used to treat certain genetic diseases or immune disorders.

However, several challenges are predictable for cell therapy–based applications using human ES cell–derived hematopoietic cells. First, for some hematopoietic cell therapies such as the development of red blood cells for transfusion, the number of cells needed is enormous. Novel bioengineering methods need to be developed in order to produce the cells on a larger scale. Second, the risk remains for development of teratomas from human ES cell–derived hematopoietic cells if any pluripotent cells are mixed in the transfusion. Thus, the safety of these cells must be strictly assessed, if these cells are to reach their therapeutic application. Finally, host immune cells may reject allergenic human ES cell–derived hematopoietic cells, and the foreign cells may also induce graft versus host disease. The last
challenge can now be overcome by using iPS cells to obtain patient-specific hematopoietic cells as described earlier for iPS cell–derived neural cells.

3.5.4 Differentiation of Hematopoietic Cells from Human iPS Cells
In 2009, the OP9 coculture system was used by the Slukvin laboratory at the University of Wisconsin to direct the differentiation of human iPS into hematopoietic progenitors (Choi et al. 2009). Although the hematopoietic differentiation potential of the human iPS cell lines is very similar to potential of human ES cell lines, there are some variations in the efficiency of hematopoietic differentiation between different human iPS cell lines. Other laboratories have also observed these differences. Advanced Cell Technologies, Inc. found that the hematopoiesis efficiency of the various iPS cell lines is much lower than that of ES cells (Feng et al. 2010). Some possible reasons for this decrease in efficiency are increased apoptosis, severely limited expansion capability, and early aging (Feng et al. 2010). It could also be caused by the differences between tissue sources, reprogramming extensiveness, etc.

3.6 MESENCHYMAL STEM CELLS
Mesenchymal stem cells (MSCs) are multipotent, and can differentiate into multiple cell types, including adipocytes, cartilage, bone, tendons, muscle, and skin. MSCs have been used in preclinical models for tissue engineering of bone, cartilage, muscle, marrow stroma, tendon, fat, and other connective tissues (Caplan 2005). MSCs have also been used to treat heart failure and acute heart infarction due to their ability to differentiate into cardiomyocytes and vascular endothelial cells and to secrete angiogenic and anti-apoptotic factors (Ohnishi and Nagaya 2007). It has also been shown that MSCs have immune suppressive function, which is being used to treat autoimmune disease (Uccelli et al. 2007).

3.6.1 MSCs Isolated from Adult Tissues
MSCs are traditionally isolated from BM (Friedenstein et al. 1966). Now, they can also be isolated from many other sources such as UCB, peripheral blood, fallopian tube, and fetal liver and lungs. However, the MSCs derived from the adult tissues have very low percentages in their source tissues, limited capacity to proliferate (Bianchi et al. 2003), and quickly have reduced multipotency and viability during aging (Kretlow et al. 2008). Thus, use of ES cells may overcome some of the limitations, perhaps permitting them to become a reliable source of cells for various therapeutic applications.

3.6.2 In Vitro Differentiation of MSC from ES Cells
MSCs can also be derived from human ES cells, which can be done in several ways. One method cocultures human ES cells with the OP9 stroma cell line (Barberi et al. 2005). Following 40 days of coculture, the cells can be harvested and sorted for CD73+ cells to purify the MSCs. Other cell surface markers such as CD105, CD90, Stro-1, CD106, CD29, CD44, CD43, and CD166 are used to further characterize the purified MSCs. Since these cells are grown in culture systems similar to that for HSC differentiation, the CD34, CD45, and CD14 markers are used to rule out the presence of HSCs. A method that can be used is
a stroma-free differentiation system, which just uses gelatin-coated plates to culture the ES cells in DMEM/F12 medium supplemented with insulin, transferrin, and selenium (ITS medium) (Barberi et al. 2007). These cells are further cultured in α-MEM to increase the percentage of MSCs. MSCs derived from both methods can be differentiated into bone, cartilage, fat, and muscle cells. In addition, ES cells can also spontaneously differentiate into MSCs on the feeder MEF (Olivier et al. 2006). These MSCs can be scraped off, replated into a new dish, and cultured in DMEM for another 4 weeks to yield a population of cells with uniform morphology. The advantage of this method is that it yields high percentage of CD73+ cells without requiring further purification.

3.6.3 Functional Differentiation of Human ES Cell–Derived MSCs
Myogenesis, osteogenesis, adipogenesis are most commonly used assays for characterization of MSCs. For myogenesis, the skeletal muscle marker NCAM is used to purify the myogenic potential of MSCs. N2 medium is used for the terminal differentiation of these cells into myocytes. For osteogenesis, β-glycerophosphate method can be used. In brief, the cells are cultured for 21 days in osteogenic medium containing dexamethasone, ascorbic acid, and β-glycerophosphate (Colter et al. 2001). For adipogenesis, both classic 3-isobutylmethylxanthine method (21 day culture in medium containing hydrocortisone, isobutylmethylxanthine, and indomethacin) (Pittenger et al. 1999) and serum withdrawal/hypoxia (SWH) methods (21 day culture in hESC medium in 5% O₂) (Olivier et al. 2006) have produced consistent results.

3.6.4 Immunosuppression of Human ES Cell–Derived MSCs
It has been shown that human ES cell–derived MSCs have even stronger immunosuppressive activity than the BM-derived MSCs, according to a recent report (Yen et al. 2009). The authors found that human ES cell–derived MSCs do not express HLA-DR and costimulatory molecules, but express HLA-G, a nonclass MHC I protein involved in mediating maternal–fetal tolerance. Human ES cell–derived MSCs can suppress CD4 and CD8 T cell proliferation, and the cytotoxic effects of NK cells. Thus, it may be possible in the future to use MSCs to treat multiple autoimmune diseases such as MS, diabetes, arthritis, Alzheimer’s, and AMS (El-Badri et al. 2004).

3.6.5 Challenges and Perspectives
Use of human ES cell–derived MSCs may have several advantages for potential clinical applications. First, unlimited number of MSCs can be generated from self-renewing human ES cells. Second, MSCs isolated from adult tissues have been widely used in clinical trial to treat many diseases, and one of the major mechanisms for the MSC activities is their capability of cytokine secretion rather than tissue regeneration. Thus, it is possible to mitotically inactivate MSCs differentiated from human ES cells and use them for clinical trials to avoid the possible tumorigenicity by residual undifferentiated human ES cells. Lastly, human ES cell–derived MSCs can also be genetically modified via gene targeting on human ES cells.
3.7 ESSENTIAL ROLE OF BIOENGINEERING IN STEM CELL RESEARCH

Despite the remarkable potential clinical applications of the stem cell types described earlier, how to best mimic the physiological environments for these stem cells or their derivatives remains a daunting question. Bioengineering has come to play as a vital approach to optimizing both \textit{in vitro} and \textit{in vivo} conditions for stem cell culture and therapy. Biomaterials approaches, in combination with other bioengineering technologies such as microfabrication and microfluidics, are well suited to assist studies of stem cell biology through the creation of evolving systems that allow key variables to be systematically altered and their influence on stem cell fate analyzed.

Specifically, bioengineering can facilitate optimization of stem cell culture condition, especially toward the development of current good manufacturing practice (cGMP) system. cGMP is a quality assurance that is used in the pharmaceutical industry to define the quality and traceability of raw materials used in validated standard operating procedures. In order for stem cell research to reach its clinical potential, everything used for stem cell research must be cGMP grade. Mechanical methods, instead of the traditional enzymatic methods, have allowed the derivation of human ES cell lines under animal-free condition. Xeno-free defined media such as the TeSR medium series and xeno-free extracellular matrices such as CellStart (which replaces the mouse tumor–derived Matrigel) have assured the complete animal-free conditions for culture of human ES/iPS cells. Nevertheless, many animal-derived and unidentified materials such as fetal bovine serum and differentiation-inducing stromal cells are still used for \textit{in vitro} differentiation of human ES/iPS cells, thus unqualified for the cGMP grade. More research is needed to develop and validate additional xeno-free materials to be used in protocols to direct the differentiation of human ES/iPS cells into specific cell types for potential therapeutic applications for patients.

3.8 CONCLUSION

In this chapter, we have discussed the regeneration of neural, hematopoietic, and mesenchymal tissues as examples to elucidate how various types of stem cells including multipotent (fetal or adult) stem cells (e.g., NSCs, HSCs, and MSCs) and pluripotent stem cells (e.g., ES and iPS cells) can differentiate into functional cells of these lineages.

3.8.1 Multipotent Stem Cells

There are many advantages to use fetal or adult multipotent stem cells in regenerative medicine. Immunocompatible autologous cells can be derived from adult stem cells. Moreover, adult stem cell can be easily differentiated into specific lineages. However, despite the ability of adult stem cells to self-renew and differentiate \textit{in vivo} throughout an entire human lifetime, scientists have not been able to expand or differentiate adult stems \textit{ex vivo} as effectively. Great effort is being made to understand and recapitulate the niches that mediate the self-renewal and differentiation of adult stem cells. Greater understanding at the molecular level of stem cell biology is needed in order to allow engineers to design better scaffolds that can retain the regenerative capacity of the stem cells and direct their fate. In addition, more cell surface markers need to be elucidated in order to isolate these rare stem cells. Another
hurdle that must be overcome is the loss of their regenerative capacity during their *ex vivo* expansion. Better culture conditions need to be developed that more closely mimic the cell–cell interactions and cell–matrix interactions found within the stem cell niches.

### 3.8.2 Pluripotent Stem Cells

The pluripotency of ES and iPS cells is a double-edge sword, which allows the cell differentiation into any lineage of therapeutic value provided the appropriate experimental protocol can be developed. On the other hand, it also potentially causes teratomas in the host if residual undifferentiated cells are mixed in the transplanted therapeutic cells. ES cells hold the golden standard for pluripotency as indicated by their differentiation ability. However, the ability of iPS cells to differentiate into neural and hematopoietic cells varies among different cell lines, and is generally lower than that of ES cells (Choi et al. 2009; Feng et al. 2010; Kim et al. 2010; Kulkeaw et al. 2010). This may result from varying levels of reprogramming of the epigenome of their parental somatic cells.

In addition, an ES cell line carries a genotype identical to that of the original embryo, so their progeny will express a HLA type irrelevant to most potential recipient patients. Whereas, an iPS cell line carries a patient-specific genome, so their progeny will express a HLA type identical to that of the donor patient. Thus, unlike ES cells, iPS cells avoid the concern of immunorejection by the recipient of their progeny.

The extremely low efficiency of iPS cell derivation via protein transduction remains a hurdle, although this best preserves the genomic integrity of the cells and avoids oncogenesis that would otherwise happen in iPS cells reprogrammed via DNA delivery. Finally, some common questions for both ES and iPS cells include how to determine and obtain ideal population(s) of therapeutic cell types for specific disease, and how to improve and achieve the survival, integration, and long-term functions of transplanted cells in the host environment. Solving these questions will certainly expedite the realization of the great promise of stem cell–based therapy.

**REFERENCES**


