Stem Cells

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Summary: Stem cells are self-renewing cells capable of differentiating into multiple cell lines and are classified according to their origin and their ability to differentiate. Enormous potential exists in use of stem cells for regenerative medicine. To produce effective stem cell–based treatments for a range of diseases, an improved understanding of stem cell biology and better control over stem cell fate are necessary. In addition, the barriers to clinical translation, such as potential oncologic properties of stem cells, need to be addressed. With renewed government support and continued refinement of current stem cell methodologies, the future of stem cell research is exciting and promises to provide novel reconstructive options for patients and surgeons limited by traditional paradigms. (*Plast. Reconstr. Surg.* 126: 1163, 2010.)

During the last decade, stem cell research has emerged at the forefront of innovative technologies poised to revolutionize medicine. Enormous potential exists in the exploitation of the body's intrinsic repair capabilities to regenerate diseased or injured organs. Stem cell research, however, has remained a highly controversial topic, and important ethical issues remain unresolved. Regardless, stem cell–based therapies continue to evolve and will play an increasing role in addressing the growing need for replacement organs and tissues worldwide.

Stem cells are defined by their capacity to both self-renew and differentiate into multiple cell lines. Traditionally, they have been divided into two main groups based on their potential to differentiate. Pluripotent stem cells (embryonic) can differentiate into *every* cell of the body, whereas multipotent stem cells (adult) can differentiate into *multiple*, but not all, cell lineages. In addition to the traditional stem cell classification, a new class of stem cells has recently been described—induced pluripotent stem cells—which are derived from genetically reprogrammed adult cells (Fig. 1). These diverse cell populations will provide researchers and clinicians with an expanded armamentarium to treat diseased and dysfunctional organs.

Reconstructive surgeons in particular stand to benefit enormously from advances in regenerative medicine. The field of plastic surgery is predicated on the intrinsic plasticity of the human body, and only recently has this plasticity been appreciated on a molecular level. Current reconstructive paradigms utilize autologous (skin, nerve, and cancellous bone grafts, myo/fascio/cutaneous flaps), allogenic (cadaveric skin and bone), and synthetic (metallic joints, nerve conduits, skin substitutes) biomaterials to restore or replace missing tissues. These approaches, however, are limited mainly by donor-site morbidity, limited supply, and/or immune reactions. Stem cell technologies promise to provide an unlimited, autologous, or nonautologous nonimmunogenic source of reconstructive material for plastic surgeons treating an ever-expanding and aging population. This review will provide an overview of current advancements in the field of stem cell research, as well as perspectives for future clinical applications.

EMBRYONIC STEM CELLS

Embryonic stem cells are derived from the inner cell mass of the blastocyst and have the capacity to differentiate into all tissues of the body.[1] Since the successful isolation of mouse and human embryonic stem cells, their potential in cell replacement therapy and regenerative medicine has

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Received for publication November 19, 2009; accepted February 19, 2010.
The first two authors contributed equally to this work.

DOI: 10.1097/PRS.0b013e3181ea42bb

Disclosure: *The authors have no financial interest in any of the products or devices mentioned in this article.*

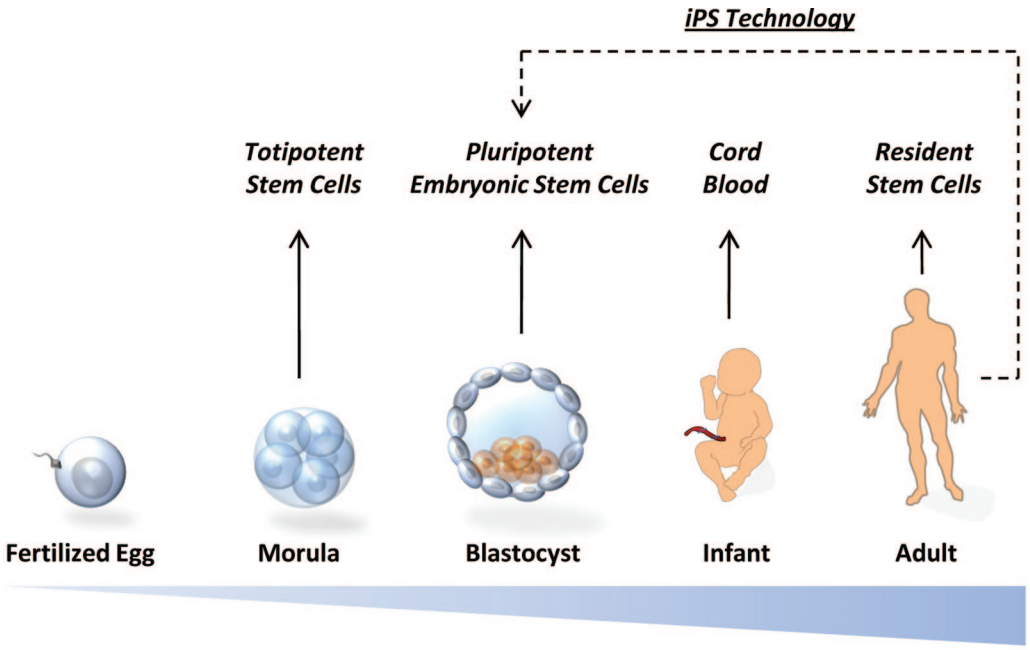

Fig. 1. Stem cells can be obtained during the different developmental stages of life. With the development of induced pluripotent stem cell technology, adult cells can now be reprogrammed to become embryonic "stem cell–like" cells.

been widely acknowledged.[2,3] Researchers have generated at least 225 human embryonic stem cell lines by placing isolated cells of the inner cell mass on human or mouse feeder layers of fibroblasts.[4] Both mouse and human embryonic stem cells have demonstrated an in vitro capacity to form cardiomyocytes, hematopoietic progenitors, neurons, skeletal myocytes, adipocytes, osteocytes, chondrocytes, endothelial cells, and pancreatic islet cells when cultured under specific growth factor conditions.[5–8]

Multiple limitations, however, currently exist regarding the use of human embryonic stem cells in regenerative medicine. Although their pluripotentiality and their unlimited ability for self-renewal make embryonic stem cells attractive for cell replacement therapy, these same characteristics simultaneously translate into unregulated differentiation and formation of teratomas and teratocarcinomas, especially in undifferentiated states (Fig. 2).[9] Without the elimination of this possibility, the clinical use of embryonic stem cell–derived tissue will remain limited. Another hurdle to embryonic stem cell–based therapies is the potential immune response to an embryonic stem cell–derived tissue graft, and concerns have been raised over the acquisition of immunogenic residues secondary to culture on mouse feeder cells.[10] Furthermore, there are significant political and ethical hurdles that hinder further investigations of human embryonic stem cells. At this time, the limited number of embryonic stem cell lines available and the restrictions placed on their use have precluded major progress in embryonic stem cell–based applications. Therefore, alternative solutions are needed to advance cell-based regenerative strategies.

SOMATIC NUCLEAR CELL TRANSFER

Somatic nuclear cell transfer, also referred to as therapeutic cloning, involves the transfer of nuclei from postnatal somatic cells into an enucleated ovum (Fig. 3). Mitotic divisions of this cell in culture lead to the generation of a blastocyst capable of yielding a whole new organism.[11] Major advances in this field came with the production of a normal sheep (Dolly), and this procedure was reproduced in other mammals, including mice, cattle, pigs, cats, and dogs.[12–17] These works suggest that same procedure might work with humans for therapeutic cloning, in which human embryonic stem cells produced by this approach could be subsequently differentiated into therapeutically useful cells and transplanted back into patients with degenerative diseases. A recent report on primate embryonic stem cell lines, which were derived from rhesus macaque somatic nuclear cell transfer blastocysts using adult male skin fibro-

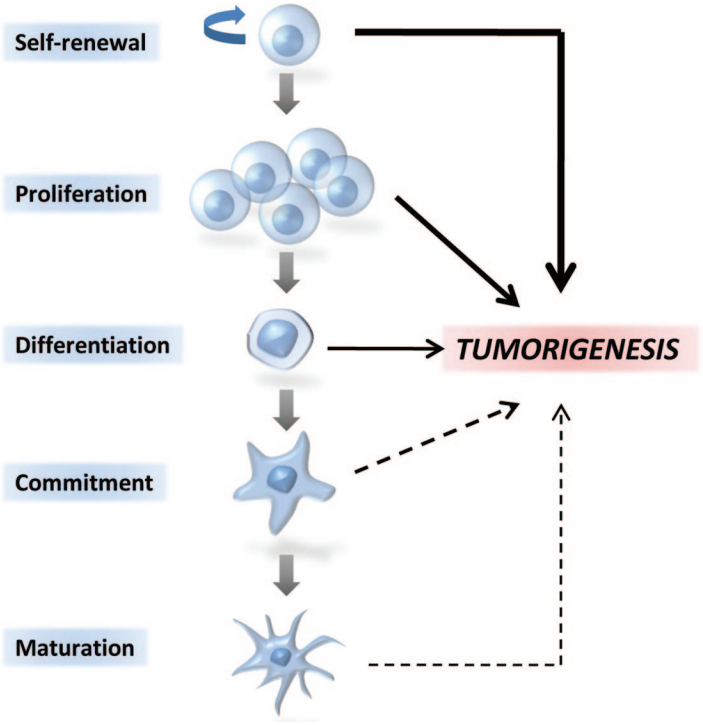

Fig. 2. During self-renewal, stem cells have an increased potential for tumorigenicity, which decreases during differentiation.

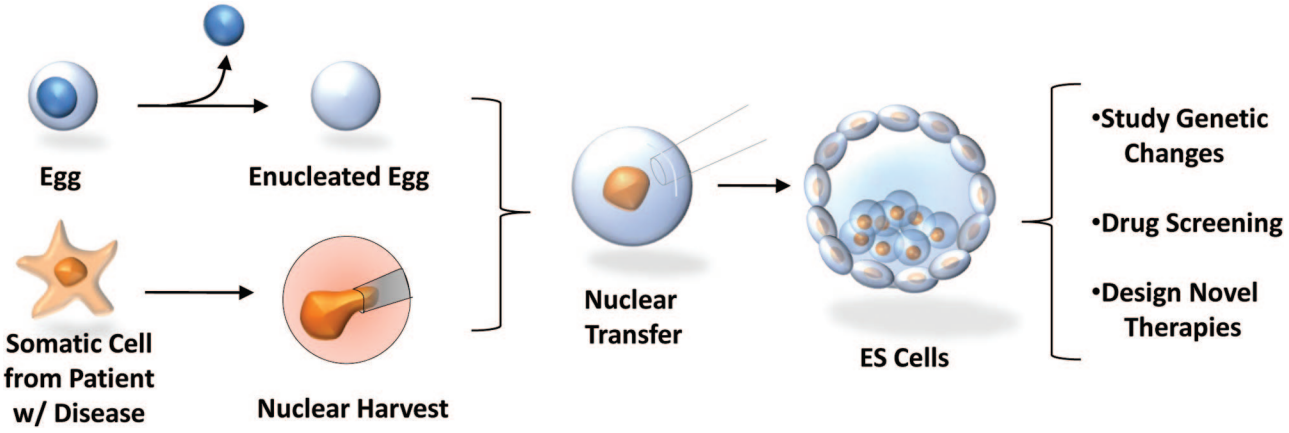

Fig. 3. Somatic nuclear cell transfer involves the transfer of nuclei from postnatal somatic cells into an enucleated ovum. In therapeutic cloning, stem cells could be derived from a patient, which would then allow the design of novel therapies.

blasts as nuclear donors, is an important step in this direction.[18]

Similar to human embryonic stem cells, however, somatic nuclear cell transfer is embroiled in an ethically complex debate about the moral status of the created embryo and concerns about obtaining human unfertilized eggs. The technical limitations of this procedure have also dampened early enthusiasm, with several studies reporting less than 10 percent efficiency in the derivation of somatic nuclear cell transfer–generated embryonic stem cells.[19] These challenges, along with the retraction of two high-profile publications that contained fabricated data on human somatic nuclear cell transfer, are some of the problems that need to be addressed.[20] Despite the controversy, somatic nuclear cell transfer may still be a promising means to generate genetically matched stem cell lines. Long-lasting cell lines from patients created via somatic nuclear cell transfer could be used

to screen potentially useful drugs or other treatments and may provide replacement cells for damaged organs in the future.

ADULT STEM CELLS

In contrast with embryonic-derived tissues, adult stem cell sources avoid the ethical concerns regarding fetal tissue harvest. For tissue-engineering purposes, a well-studied adult stem cell population includes mesenchymal stem cells (Fig. 4). Among a number of definitions that exist, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy defined mesenchymal stem cells by the following characteristics[21,22]:

1. They are plastic-adherent when maintained in standard culture conditions.
2. They express the surface markers CD73/CD90/CD105 and lack expression of CD14/CD34/CD45, CD11b/CD79, and CD19/HLA-DR.
3. They are capable of differentiation into osteoblasts, chondrocytes, and adipocytes in vitro.

Mesenchymal stem cells have been isolated from bone marrow,[23–25] umbilical cord blood,[26] and adipose tissue.[27,28] The tissue origin of mesenchymal stem cells seems to be a major determinant of progenitor characteristics. For example, colony-forming frequency was lowest in stem cells derived from umbilical cord blood and highest in adipose tissue–derived stem cell populations, although the proliferation capacity was highest in umbilical cord blood stem cells.[29]

Although mesenchymal stem cells can be obtained from diverse sources, adipose tissue–derived stem cells in particular fulfill several requirements proposed for successful clinical use in regenerative applications[30]:

1. They should be found in abundant quantities (up to billions of cells).
2. They should be harvested with a minimally invasive procedure.
3. They should be able to differentiate along multiple cell lineage pathways in a controllable and reproducible manner.
4. They should be safely and effectively transplanted into an autologous or allogeneic host.
5. They can be manufactured in accordance with good manufacturing practice guidelines.

Adipose tissue–derived stem cells can be readily harvested during a minor liposuction pro-

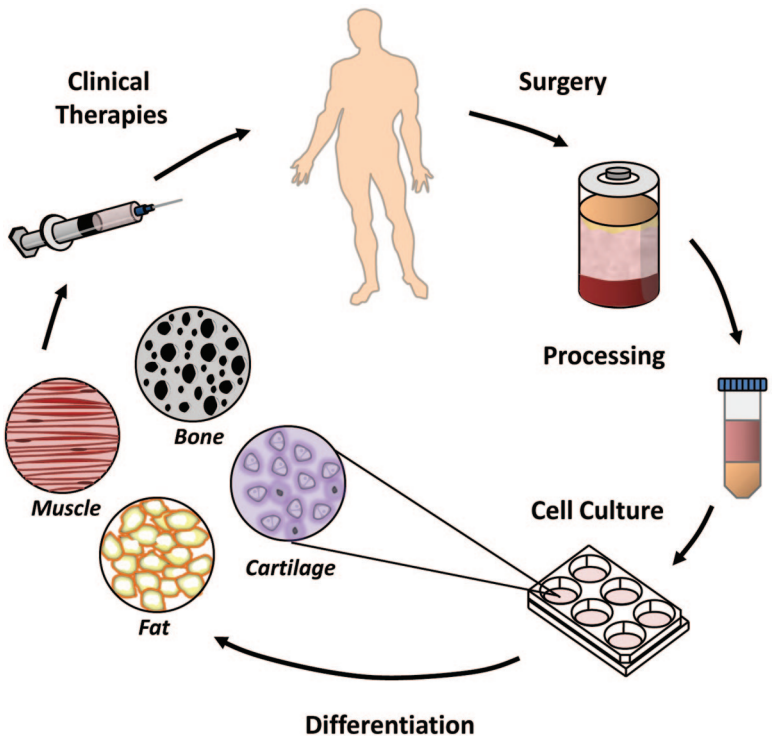

Fig. 4. Adipose-derived stem cells in a reconstructive cycle. Stem cells can be harvested by liposuction, isolated, and differentiated. They could then be autologously transplanted back into the patient.

cedure under local anesthesia, in contrast with the significant pain experienced by patients undergoing bone marrow stem cell harvest from the iliac crest. Undoubtedly, plastic surgeons are in the ideal position to capitalize on this technology, being able to both harvest and reimplant tissue from this source.

Adipose tissue–derived stem cells have been successfully used in regenerative applications in numerous animal models and have demonstrated the capacity to differentiate into cartilage, bone, muscle, and adipose tissue. For example, they have been shown to facilitate chondrogenesis in a rabbit condylar defect model.[31] Their osteogenic potential has been demonstrated by our laboratory in a murine calvarial defect model.[32] Adipose tissue–derived stem cells seeded onto apatite-coated poly-lactic-co-glycolic acid scaffolds were implanted into 4-mm critical-sized calvarial defects and demonstrated improved healing compared with non–adipose tissue–derived stem cell–seeded implants. In addition, their myogenic capability has been shown in a Duchenne muscular dystrophy mouse model.[33] Human adipose tissue–derived stem cells were injected into the tibialis anterior muscles of dystrophin-deficient mice, and after 6 months, 90 percent of the myofibers were dystrophin positive, indicating substantial integration of the human cells into the muscle. Finally, they have also exhibited adipogenic potential in several murine models.[34,35]

TISSUE-SPECIFIC STEM CELLS

In addition to embryonic stem cells and mesenchymal stem cells, tissue-specific "resident" stem cells have been identified in almost all postnatal tissues and organs.[36] Tissue-specific stem cells reside in niches, whereby complex microenvironmental cues maintain their multipotency.[37] They are capable of both self-renewal and differentiation throughout the lifespan of an individual and utilize both mechanisms to maintain a steady state and regenerate injured tissue.[36,38] Disruptions in stem cell–regulated homeostasis are even hypothesized to result in tumorigenesis.[39] Researchers are actively investigating the potential to stimulate resident stem cell differentiation in vivo to assist in tissue repair. In addition, transplantation of exogenous tissue resident stem cells may provide the cellular stimulus needed to drive local regeneration processes.

As an example, the role of resident stem cells has been well-described in skin, an organ of particular interest to the plastic surgeon.[40] Positioned at the interface between the human body and the external world, epithelial tissues are constantly exposed to injury and require complex mechanisms for both self-renewal and repair. Distinct lineages of skin stem cells have been described in the basal epithelium, hair follicles, and sebaceous glands.[40] Basal epithelial stem cells are proposed to undergo both *asymmetric* division (dividing into one stem cell for self-renewal and one differentiated daughter cell for epidermal stratification) and *symmetric* division (dividing into either two stem cells or two differentiated daughter cells) (Fig. 5).[41] This complex differentiation system is further regulated by epigenetic factors, such as cytokines and growth factors, and a thorough understanding of these mechanisms will be critical for stem cell–based therapies aimed at skin regeneration.

INDUCED PLURIPOTENT STEM CELLS

Takahashi and Yamanaka published a landmark article in 2006 that defined a specific set of transcription factors capable of reverting differentiated cells back into a pluripotent state, thus creating "induced" pluripotent stem cells (Fig.

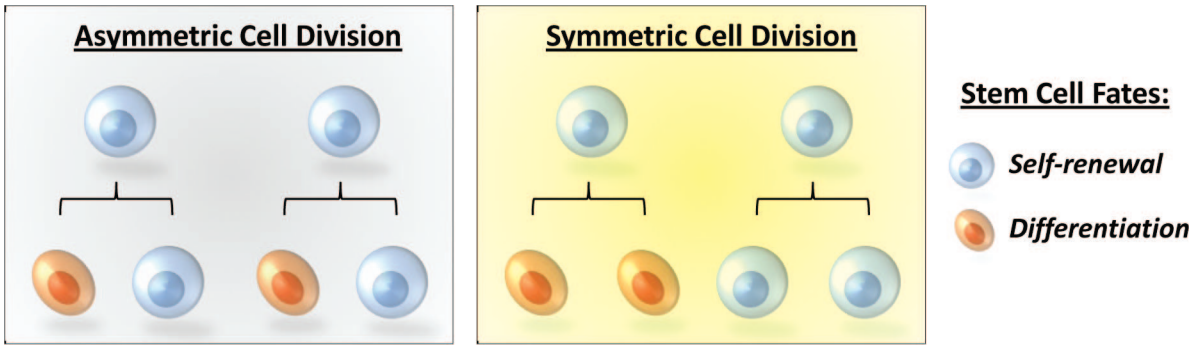

Fig. 5. Asymmetric division of epithelial stem cells is defined by the division of one stem cell for self-renewal and another differentiated daughter cell for epidermal stratification, whereas symmetric division results in two stem cells or two differentiated daughter cells.

6).[42] Four key transcription factors—Oct4, Sox2, Klf4, and c-Myc—identified by screening 24 preselected murine embryonic stem cell–specific factors were sufficient to reprogram adult mouse fibroblasts into embryonic stem cell–like induced pluripotent stem cells.[42] The same combination of transcription factors has been demonstrated to be sufficient for pluripotent induction of human cells as well.[43] Considering the ease and reproducibility of generating induced pluripotent stem cells compared with somatic nuclear cell transfer, experts have raised the hope that induced pluripotent stem cells might fulfill much of the promise of human embryonic stem cells in regenerative medicine.[44]

It is widely accepted that mouse and human induced pluripotent stem cells closely resemble molecular and developmental features of blastocyst-derived embryonic stem cells.[42,45,46] Different research groups have shown that induced pluripotent stem cells injected into immunodeficient mice give rise to teratomas comprising all three embryonic germ layers, similar to embryonic stem cells. In addition, when injected into blastocysts, induced pluripotent stem cells generated viable high-contribution chimeras (mice that show major tissue contribution of the injected induced pluripotent stem cells in the host mouse) and contributed to the germline.[42,45,46] Furthermore, using reverse transcriptase polymerase chain reaction and immunocytochemistry, studies have shown that induced pluripotent stem cells express key markers of embryonic stem cells.[42,45,46] Additional studies, however, need to be done to address whether induced pluripotent stem cells are indeed identical to embryonic stem cells. Global gene expression analysis comparing induced pluripotent stem cells with human embryonic stem cells using microarrays demonstrated that approximately 4 percent of the more than 32,000 analyzed genes had a greater than five-fold difference in expression.[43] Furthermore, chimeras and progeny mice derived from induced pluripotent stem cells had higher than normal rates of tumor formation than those derived from embryonic stem cells, which in some cases may be due to reactivation of the transfected c-Myc oncogene.[47] These key differences need to be further elucidated to define the safety of induced pluripotent stem cell use in regenerative medicine.

Another potential complication with the generation of induced pluripotent stem cells is the use of retroviral and lentiviral vectors to activate the necessary reprogramming transcription factors. Specifically, the risk of insertion mutagenesis could lead to uncontrolled modification of the genome.[48] Much progress has been made in generating integration-free murine induced pluripotent stem cells, and recent various studies using adenoviral, plasmid-based, and recombinant protein-based strategies have reported that viral integration is not required for the reprogramming process.[49–51] Even without viral integration, the safety of induced pluripotent stem cells needs to be rigorously tested, since all essential reprogramming factors are oncogenes and their overexpression has been linked with cancers.[52] The characterization of induced pluripotent stem cells will be enhanced by ongoing improvements in high-resolution analysis of genomic integrity

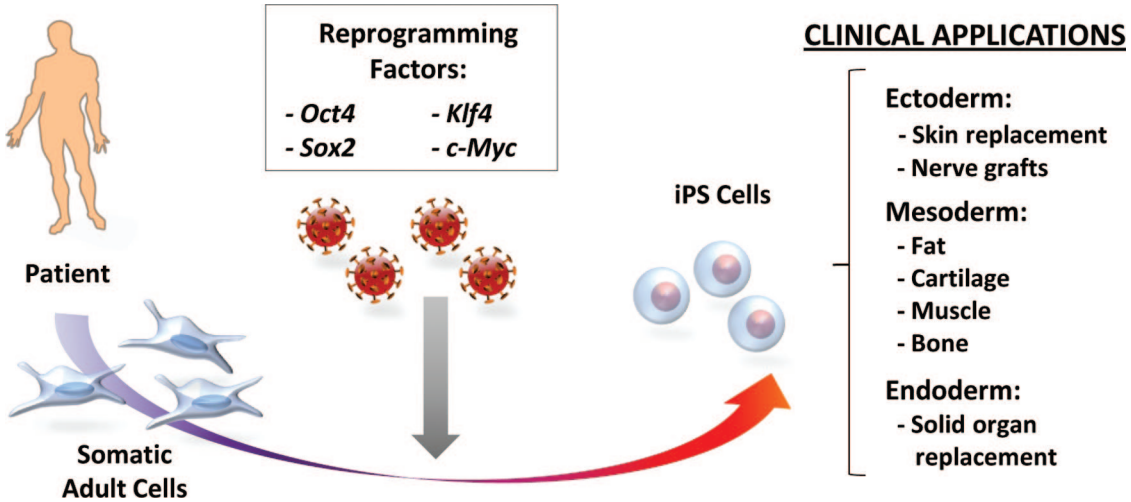

Fig. 6. By activation of four transcription factors, adult somatic cells can be reprogrammed into induced pluripotent stem cells, which then can differentiate into all the embryonic lineages: ectoderm, mesoderm, and endoderm.

via DNA sequencing technology to readily identify even minor deletions, inversions, or loss of individual alleles.[53]

Generation of induced pluripotent stem cells is likely to create a major impact on regenerative medicine. Induced pluripotent stem cells can be generated from human adipose tissue–derived stem cells in a feeder-free condition with a faster speed and higher efficiency than comparable strategies targeting adult human fibroblasts.[54] Given the ease of isolating a large quantity of these cells from lipoaspirates, adipose tissue–derived stem cells could be an ideal autologous source of cells for generating individual-specific induced pluripotent stem cells. Induced pluripotent stem cells have already been differentiated into various functional cell types, including hematopoietic cells and neurons.[55–57] Therapeutic potential of induced pluripotent stem cells has been demonstrated in several preclinical models. For example, Wernig et al. demonstrated that neurons derived from reprogrammed fibroblasts could alleviate the disease-phenotype in a rat model of Parkinson's disease.[57] Using a humanized sickle cell anemia mouse model, Hanna et al. showed that the genetic defect was corrected using induced pluripotent stem cells derived by coupling reprogramming and homologous recombination of intact wild-type β-globin gene.[56] Although these early preclinical studies are very promising, induced pluripotent stem cell technology will require further refinement before clinical applications can be feasible.

CLINICAL APPLICATION OF STEM CELLS

Stem cell–based clinical trials are still in the early stages of development. In a preliminary case study, three patients were treated with autologous bone marrow stem cells seeded onto porous ceramic scaffolds for limb cortical defects ranging from 4 to 7 cm.[58] The patients were monitored for up to 27 months, and callus formation with graft integration was evident after 2 months as compared with 12 to 18 months if traditional methods were applied. A long-term follow-up study from the same patient group revealed no major complications up to 7 years after surgery.[59] In another case report, a 7-year-old girl with a critical-sized calvarial defect was successfully treated with cancellous iliac bone grafts in combination with autologous adipose tissue–derived stem cells.[60] In a case series, 20 patients with severe symptoms or irreversible functional skin damage due to radiotherapy were treated with autologous adipose tissue–derived stem cells delivered by computer-assisted injections.[61] A clinical improvement was reported in all patients treated with the cells.

Interestingly, the first clinical phase I trial for utilizing adipose tissue–derived stem cells was carried out in patients with Crohn's disease.[62] In this trial, 75 percent of nonhealing fistulas (six of eight total fistulas in five patients) unsuccessfully treated with traditional therapies showed complete healing with adipose tissue–derived stem cell–based treatment. In a consecutive multicenter phase II trial, 50 patients with perianal fistulas were randomized to treatment with fibrin glue combined with 20 million adipose tissue–derived stem cells or fibrin glue alone.[63] In the stem cell–treated group, 71 percent healing was observed in comparison with 16 percent in the control group. Although the contribution of adipose tissue–derived stem cells in the healing process was only assessed clinically, these data still indicate a potential benefit in the stem cell–based treatment of perianal fistulas. In another phase I clinical trial, eight patients with Duchenne disease were treated with myogenic (muscle-derived $CD133^+$) stem cells.[64] Although no systemic side effects were noted, there were also no differences in outcome between treatment and control patients.

Almost 10 years after the first human embryonic stem cells were isolated at the University of Wisconsin, the U.S. Food and Drug Administration approved the world's first clinical trial with human embryonic stem cells in early 2009. The study, which is being conducted by Geron, a California-based biotech company, is aimed at treating acute spinal cord injury patients and based on promising data obtained in a rat model.[65] This trial is a milestone in the early history of human stem cell research and signals a new era of government support for stem cell research.

CONCLUSIONS

Stem cell technology has flourished as an exciting field encompassing almost every organ and tissue system. Current concepts of stem cell biology have provided much insight into the physiological and pathological states of tissue regeneration. Although exciting progress has been made in studying embryonic stem cells and induced pluripotent stem cells, their potentiality and differentiation must be better harnessed before widespread clinical use. On the other hand, various forms of adult stem cells are thought to be safer at the expense of decreased differentiation potential. Researchers have only started to translate this

scientific knowledge to clinical application, but stem cell technologies promise to provide innovative tools for plastic surgeons in their attempt to reconstruct the human body.

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GLOSSARY OF TERMS

Totipotency: Ability of a cell to form a complete organism, including extraembryonic tissue (yolk sac, placenta).

Pluripotency: Ability of a cell to form a complete organism. Embryonic stem cells are pluripotent and derived from the inner cell mass of blastocysts.

Multipotency: Ability of a cell to form cell types of one embryonic lineage such as the mesoderm. Most adult stem cells are multipotent.

Embryonic stem cells: Cells derived from the inner cell mass of a blastocyst, which are pluripotent and can give rise to many different cell lineages and cell types.

Adult stem cells: Mesenchymal cells that are found in bone marrow, adipose tissue and umbilical cord blood, as well as resident stem cells, which can be found in almost any organ of the body. Adult stem cells are multipotent.

Induced pluripotent stem cells: Adult cells that, when exposed to transcription factors, get reprogrammed to cells with embryonic stem cell like properties.

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