Stem Cell Platforms for Regenerative Medicine

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Abstract

The pandemic of chronic degenerative diseases associated with aging demographics mandates development of effective approaches for tissue repair. As diverse stem cells directly contribute to innate healing, the capacity for *de novo* tissue reconstruction harbors a promising role for regenerative medicine. Indeed, a spectrum of natural stem cell sources ranging from embryonic to adult progenitors has been recently identified with unique characteristics for regeneration. The accessibility and applicability of the regenerative armamentarium has been further expanded with stem cells engineered by nuclear reprogramming. Through strategies of replacement to implant functional tissues, regeneration to transplant progenitor cells or rejuvenation to activate endogenous self-repair mechanisms, the overarching goal of regenerative medicine is to translate stem cell platforms into practice and achieve cures for diseases limited to palliative interventions. Harnessing the full potential of each platform will optimize matching stem cell-based biologics with the disease-specific niche environment of individual patients to maximize the quality of long-term management, while minimizing the needs for adjunctive therapy. Emerging discovery science with feedback from clinical translation is therefore poised to transform medicine offering safe and effective stem cell biotherapeutics to enable personalized solutions for incurable diseases.

Keywords: embryonic, adult, perinatal, bioengineered, immune response, allogeneic, autologous

Introduction

Naturally propagated throughout development, stem cells provide the foundation for *de novo* tissue formation.[1] Embryonic stem cells (ESCs) are the prototype of progenitor cells capable of giving rise to all tissue types of the body.[2] Various forms of progenitors survive throughout adulthood and continuously contribute to tissue rejuvenation, most notably in tissues with high turnover rates.[3,4] Induction of stem cells from adult somatic tissues potentially offers additional pools of endogenous sources to respond to excessive tissue damage and augment wound healing.[5] Furthermore, proper regulation of stem cell growth and differentiation is increasingly recognized to contribute to the overall balance of health and disease throughout the aging continuum and offers pathways to target new therapeutic strategies.[6,7]

Specialized tissues derived from a wide spectrum of progenitor cells after embryonic development are required for organ rejuvenation throughout lifespan and contribute to normal healthy aging.[8–10] Asymmetrical cell division provides the fundamental principle that enables long-term survival of stem cell pools to produce daughter cells capable of transiently expanding prior to tissue differentiation, while maintaining self-renewing cell phenotype with unique gene expression patterns to preserve original features of stem cells.[11,12]

The goals of regenerative medicine are to utilize endogenous as well as exogenous natural or bioengineered stem cells to augment innate healing processes in order to supplement repair deficiencies.[13,14] Stem cell platforms offer a continuum of potential lineages ranging from pluripotent to oligopotent cytotypes. Pluripotent stem cells can give rise to all germ layers, and subsequently all adult tissues in the body, providing a universal tool for regeneration.[15] Alternatively, oligopotent progenitors offer a more limited scope of differentiation depending on a particular tissue-specific environment, and thereby provide a focused approach to repair.[16,17] Matching the needs of the specific disease condition with the unique abilities of selected progenitor cells has emerged as the critical challenge for effective translation of discovery science into safe clinical applications.[18]

Thus, stem cells are isolated from either natural sources or bioengineered from non-reparative tissues to provide a spectrum of progenitor cells with unique features for diverse regenerative applications (*Table 1*). Examples of naturally derived stem cells include ESCs, perinatal stem cells (e.g., umbilical cord-derived stem cells), and adult stem cells (e.g., bone marrow-derived stem cells). Nuclear engineering processes have enabled the derivation of stem cells by converting somatic donor cells into pluripotent progenitors. The characteristics of each platform determine the potential for safe and effective therapeutic application. Exchange of knowledge and experience garnered among diverse stem cell platforms will continue to drive the next generation of regenerative technology beyond experimental science and into clinical reality for a wide range of currently incurable disease processes.

Embryonic Stem Cells

Embryonic stem cells are derived from the inner cell mass of a preimplantation blastocyst (*Figure 1*).[1] These pluripotent stem cells can give rise to all tissues, including the complete spectrum of mesoderm, endoderm, and ectoderm derivatives (*Table 2*). Multiple ESC lines have been derived from a wide range of species, ranging from mouse to human.[19,20] Human ESCs were first isolated a decade ago, and have now been demonstrated to give rise to various cell types, including hematopoietic cells, neuron-like cells, glial progenitors, dendritic cells, hepatocytes, pancreatic islet-like cells, osteocytes, chondrocytes, adipocytes, cardiomyocytes, as well as muscular, endothelial, skin, lung, and retinal tissues. This quintessential differentiation potential provides a promising avenue to produce a large quantity of transplantable cells from a renewable source.

ESCs are defined by their unique capacity to self-renew indefinitely in cell culture, long telomere length, high nuclear to cytoplasmic ratio, and demonstrate an unparalleled differentiation capacity. The transcription factor Oct 4 coupled with Sox2 and E1A contribute to maintenance of the undifferentiated state through fibroblast growth factor-4 (FGF-4), Wnt, and transforming

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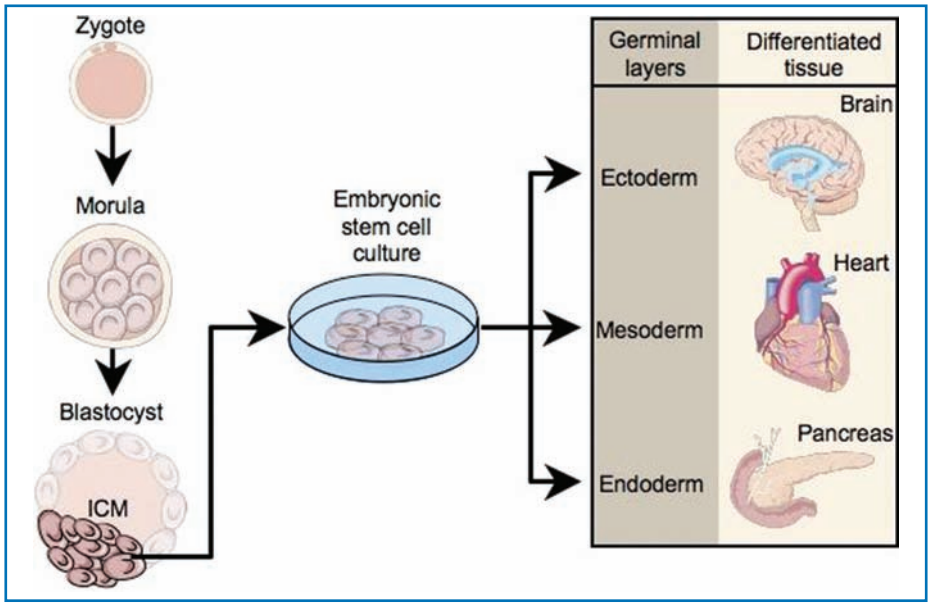

Figure 1. Embryonic stem cells. Isolated from the inner cell mass (ICM) of a blastocyst, embryonic stem cells are cultured to produce cell lines that can be indefinitely propagated in the undifferentiated state. Consistent with the characteristic ability to recapitulate normal embryonic development, embryonic stem cells differentiate *in vitro* to produce tissues arising from all three germinal layers. The ectoderm develops into neuronal derivatives within the central nervous system. The mesoderm produces mature tissues, such as heart muscle. The endoderm differentiates into lineages such as pancreas. The pluripotency and unlimited proliferation makes embryonic stem cells an attractive source for regenerative medicine applications.

Stem cells platforms	Properties
Embryonic	• Blastocyst derived
	• Pluripotent
	• Highly malleable
Perinatal	• Isolated from perinatal sources
	• Combines embryonic-like and adult-like stem cell pools
	• Abundant at birth
Adult	• Obtained from multiple tissues
	• Multipotent
	• Includes hematopoietic and mesenchymal progenitors
Bioengineered	• Produced from somatic sources
	• Utilizes therapeutic cloning or nuclear reprogramming
	• Generates customized embryonic-like stem cells

Table 1. Stem cell platforms.

Spectrum	Developmental aptitude
Pluripotent	Ability to form all lineages of the body
Multipotent	Ability to form multiple cell types within a specific lineage
Unipotent	Ability to form a single cell type

Table 2. Stem cell differentiation spectrum.

growth factor-β (TGF-β)-dependent pathways.[15] Pluripotency in mouse ESC has been maintained with leukemia inhibitory factor through a STAT-3-dependent mechanism, which is not evolutionary conserved in human counterparts. Pluripotency in human ESC is dependent on an Src family of nonreceptor tyrosine kinases, and is monitored by expression of markers such as alkaline phosphatase, POU transcription factor Oct3/4, Nanog, Cripto/TDGF1, proteoglycans TRA-1-60/81, GCTM-2, and embryonic antigens SSEA-3 and SSEA-4. Early lineage commitment of the three germinal layers has been monitored by the expression of markers such as Sox1 for neuroectoderm, Pdx-1 for endoderm, and Flk-1 for mesoderm.

A potential limitation of ESCs with regard to regenerative applications relates to their inherent unrestricted growth potential with the associated risk of teratoma formation prominent once differentiation cues are evaded following transplantation.[20] Significant progress in understanding embryonic developmental pathways has established strategies to guide ESC into tissue-specific lineages. Ensuring proper regulation of ESC differentiation prior to transplantation or within the microenvironment of transplanted host tissue provides safety required for clinical application.[21,22] Guidance of targeted differentiation using growth factors, cytokines, hormones, and small molecules provides a pharmacological approach to restrict lineage potential and promote lineage specification. Specialized biomarkers that identify tissue-specific commitment and allow physical separation from undifferentiated progenitor cells provide an additional approach to secure lineage specification.[23]

Beyond the unrestricted growth potential, ESCs create a unique immunological challenge for regenerative medicine.[24] Since ESCs are derived from nonself tissues, adult engraftment of this cell type is considered an allogeneic transplantation. Despite this allogeneic identity, ESCs are capable of engraftment into nonidentical hosts with minimal immunosuppression. Expression of MHC-1 protein is very low in ESC, and is thought to secure survival following allogeneic transplantation by avoiding immunological detection. This has provided a basis to interpret tolerance of xenotransplanted ESC-derived cardiomyocytes. Other lines of evidence suggest the feasibility of ESC-derived dendritic cells that may provide the ability to invoke immunological tolerance of engrafted tissues.[25] Antigen presenting cells from the same individual as the graft tissue allows the host to be retrained as to the definition of "self." Clinically this has been observed in patients that have undergone bone marrow transplantation prior to solid organ transplantation from the same donor and require little immunosuppressive therapy to maintain the allogeneic solid organ. Additionally, ESC-derived tissues have demonstrated a paracrine mechanism to evade immunological recognition through ESC-based production of TGF-β that locally inhibits endogenous T-cell function.[26] Alternative approaches to avoid immunological detection include genetic modifications of ESC

to eliminate MHC proteins or express immunosuppressive agents through transgenic manipulations of ESC lines. Such engineered stealth-like "universal stem cell line" would assure long-term tolerance in a foreign host. Furthermore, HLA-isotyped ESC cell lines produced and characterized to generate a cell bank could provide an "off-the-shelf" product that can be cross-referenced with the immunological requirements of individual patients to secure an appropriate stem cell source for therapy.

Given these considerations, therapeutic applications of ESC have so far been limited to preclinical studies. This includes neurological disorders such as Parkinson's disease and spinal injury,[27] endocrine disorders such as type 1 diabetes,[28] and cardiovascular disease.[29–31] In addition to structural replacement of diseased tissues, ESCs have the ability to overcome metabolic deficiencies, deliver trophic support to host cells, and restore disconnected cellular interactions.[32] These paracrine effects may prove to be a critical component to the regenerative capacity of ESC in models of human disease, providing future avenues for regenerative medicine.

Perinatal Stem Cells

There is growing evidence to support the diverse differentiation capacity of stem cells derived from perinatal sources, including umbilical cord blood (UCB). Umbilical cord blood is collected at the time of birth, and provides a pool of embryonic-like and adult-like stem cells (*Figure 2*).[33] Transplantation of UCB has been clinically successful for hematopoietic stem cell applications resulting in high degree of engraftment, favorable immunotolerance, and limited evidence for graft-versus-host disease compared to adult bone marrow stem cell transplantation. Reconstitution of the adult hematopoietic system in siblings or in the same individual was the initial rationale for UCB-based applications. In addition to hematopoietic stem cells, UCB-derived stem cells are capable of *in vitro* expansion, long-term maintenance, and differentiation into representative cells of all three embryonic germinal layers, that is, the endoderm (e.g., hepatopancreatic precursor cells, mature hepatocytes, and type II alveolar pneumocytes), mesoderm (e.g., adipocytes, chondrocytes, osteoblasts, myocytes, and endothelial cells), and ectoderm (e.g., neurons, astrocytes, and oligodendrocytes).[33] Heterogeneous stem cells contained in UCB can be fractionated to purify a more homogeneous population with characteristics of embryonic-like stem cells. In this way, UCB may provide a clinically applicable pluripotent stem cell pool that avoids ethical challenges raised with embryonic sources.[34] Alternatively, amniotic epithelial cells (AECs) derived from amniotic membranes can also be induced to differentiate into diverse and specialized cell types from all three germ layers including pancreatic cells (endoderm), cardiomyocytes (mesoderm), and keratinocytes (ectoderm). Like UCB-derived cells, AEC are derived from preexisting tissue that is readily available at the time of birth.[35] This creates an opportunity to generate alternative multilineage stem cells from nonembryonic sources.

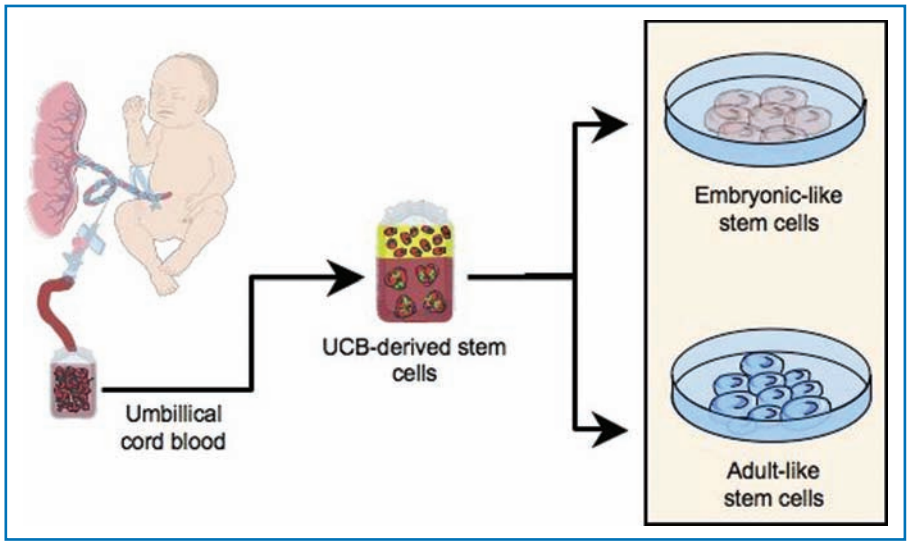

Figure 2. Perinatal stem cells. Umbilical cord blood is one source of perinatal stem cells collected at birth from the umbilical vein containing blood returning from the placenta. These cells have been utilized as hematopoietic stem cells similar to adult bone marrow-derived progenitors. The immaturity of the immune system reduces the risk of detrimental graft-versus-host disease when comparing perinatal to adult sources. Perinatal stem cells also contain populations of cells that behave similar to embryonic stem cells. Pluripotent differentiation potential and unique immunologic status highlight the advantages of perinatal stem cells for regenerative applications, and justify a distinct classification. Perinatal stem cells that also include amniotic epithelial cells (AECs) offer readily available, alternative sources of progenitors.

Adult Stem Cells

Adult stem cells comprise a wide range of progenitors derived from nonembryonic, nonfetal tissues such as bone marrow, adipose tissue, and resident stem cell pools.[36] Adult stem cells are a leading candidate for clinical application in regenerative medicine based on accessibility, autologous status, and favorable proliferative potential.[37] While there is increased understanding of the regenerative potential of stem cells derived from adipose tissue and of resident stem cells linked to the innate repair potential of mature tissues, current experience primarily relies on bone marrow-derived stem cells. Sufficient to recapitulate the entire hematopoietic system and provide mesenchymal stem cells with nonhematopoietic differentiation potential, bone marrow-derived stem cells are a cornerstone of contemporary regenerative medicine applications.[16]

Bone marrow-derived hematopoietic stem cells represent the earliest example of cell-based regenerative medicine, pioneered to address the needs of patients treated with total body irradiation for leukemia that developed life-threatening infections and irreversible tissue destruction. Stem cells defined by expression of the CD34 surface marker can also be obtained via peripheral blood leukapheresis for clinical engraftment. Hematopoietic stem cells have provided the foundation for autologous and allogeneic stem cell transplantation, and offer novel treatments for patients with cancer, autoimmune diseases, and genetic diseases, including severe combined immunodeficiency and thalassemia. Transplant studies have also revealed engraftment of nonhematopoietic cell lineages derived from donor bone marrow, unmasking subpopulations capable of a diverse range of lineage-specific differentiation.

Mesenchymal stem cells were also discovered in the bone marrow, although at low frequency compared to the hematopoietic pool. Mesenchymal stem cells represent approximately 1 out of 10,000 nucleated cells and arise from the supporting architecture of the adult

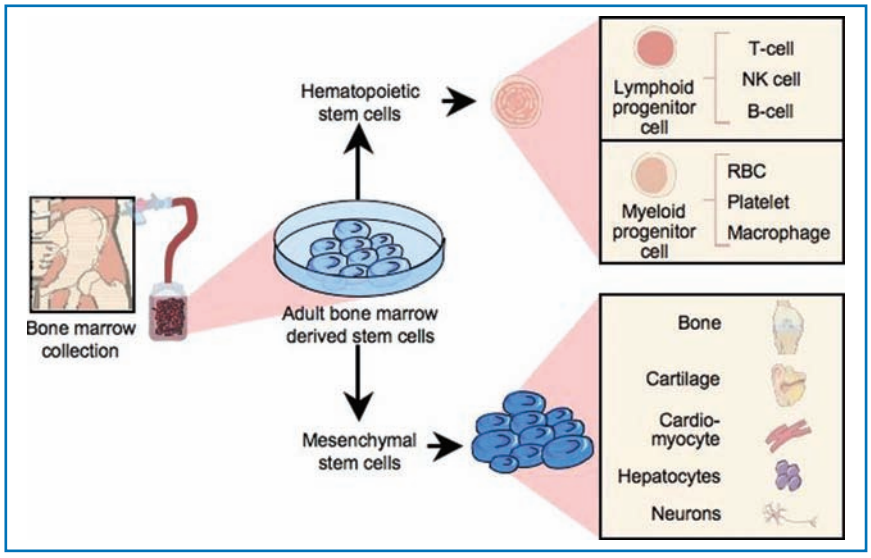

Figure 3. Adult stem cells. Derived from nonembryonic or nonperinatal sources, adult stem cells can be procured from a range of tissues, such as bone marrow, as well as circulating blood or fat. Adult stem cells are generally considered multipotent, as illustrated for bone marrow-derived stem cells that contain both hematopoietic progenitors and mesenchymal stem cells. Hematopoietic stem cells give rise to (i) lymphoid-derived T cells, B cells, and natural killer cells; and (ii) myeloid-derived red blood cells, platelets, and macrophages. Hematopoietic stem cells provide the standard of care for bone marrow reconstitution. Mesenchymal stem cells also have a diverse spectrum of differentiation that includes bone, muscle, cartilage, cardiomyocyte, hepatocyte, and neurons. Mesenchymal stem cells serve as a cell type of choice for clinical applications of nonhematological regeneration based on the availability of autologous stem cell sources, cost-effective isolation, and safety profile.

marrow.[38] Comparable stem cells have also been isolated from connective components of various postnatal tissues including adipose and synovial tissue, as well as from peripheral and cord blood. Mesenchymal stem cells exhibit properties of multipotency, with the capacity to contribute to regeneration of bone, cartilage and muscle, tissues of mesodermal origin (*Figure 3*).[39] Evidence also supports the contribution of mesenchymal stem cells to liver and pancreatic islet cell regeneration, and protection in the setting of kidney, heart, or lung injury. Mesenchymal stem cells secrete a spectrum of bioactive molecules that provide a regenerative microenvironment to limit the area of damage and to mount a self-regulated regenerative response. The age of the individual, the extent of tissue damage, and the local and whole body titers of mesenchymal stem cells have been all considered to play a role in the ultimate rate and extent of repair. Beyond the supply of differentiated mesenchymal tissues in case of cell loss, mesenchymal stem cell progeny constitutes the stromal environment that is fundamental in the regulation of parenchymal stem cell renewal and differentiation, and immune modulation. In fact, both autologous and allogeneic mesenchymal stem cells have been tested in recent clinical trials including for treatment of osteogenesis imperfecta, Crohn's disease, and graft-versus-host disease.[40]

Identification of adult human mesenchymal stem cells as multilineage progenitors relies on their capacity to be induced to differentiate into specific phenotypes. Although the ganglioside GD2 has been proposed as a single surface marker that distinguishes mesenchymal stem cells, a panel of biomarkers has been typically employed to secure isolation.[41] These include positive expression of CD44, CD73, CD90, CD105, Stro-1, and the adhesion molecules CD106 (vascular adhesion molecule VCAM-1) and the intercellular adhesion molecule ICAM-1, in the absence of expression of hematopoietic markers CD34, CD45, CD11, and CD14, and the adhesion molecule CD31 (platelet/endothelial cell adhesion molecule PECAM-1).[38–40]

Adult human mesenchymal stem cells are characteristically devoid of human leukocyte antigen (HLA) class II antigens (MHC-II) on the cell surface, and do not express the co-stimulatory molecules CD80, CD86, or CD40.[38] The expressed major histocompatibility complex (MHC) class I antigens may activate T cells, but with the absence of co-stimulatory molecules, a secondary signal would not engage, leaving T cells anergic. Such unique immune profile, that is, MHC I^+, MHC II^-, $CD40^-$, $CD80^-$, $CD86^-$, is regarded as nonimmunogenic, and accordingly transplantation into an allogeneic host may not require immunosuppression.[38] Moreover, mesenchymal stem cells exhibit immunosuppressive properties, modulating T-cell functions including cell activation, and display immunomodulatory features impairing maturation and function of dendritic cells and inhibiting human B-cell proliferation, differentiation, and chemotaxis. However, rejection of marrow stromal cells in MHC class I and class II-mismatched recipients has been reported underscoring the relevance of the immune response in the outcome of stem cell therapy. As mesenchymal stem cell interaction with the microenvironment could in principle trigger (re)expression of MHC II antigens compromising the fate of implanted cells, additional studies are needed to optimally translate mesenchymal stem cell therapy in the clinical setting. An increased understanding of how mesenchymal stem cells regulate the inflammatory response, distribute, and differentiate after delivery in humans is needed.[42]

At present, the consensus favors use of allogeneic mesenchymal stem cell therapy in the management of acute disorders, such as acute myocardial infarction, acute graft-versus-host disease and acute exacerbations of inflammatory bowel disorders. This strategy avoids the need for preparing autologous cells from the recipient. For disorders where mesenchymal stem cells are not needed on an emergency basis, it may be preferable to culture-expand autologous mesenchymal stem cells prior to implantation in an effort to personalize cell-based therapy.[42] Expansion offers the opportunity to produce a large pool of naïve stem cells, and derive progeny honed for lineage-specification away from multipotency and into tissue-restricted cytopoiesis.[37] Derivation and characterization of specialized mesenchymal stem cell subpopulations, and their selective application to specific disease conditions, is an emerging strategy for enhanced therapeutic outcome.

Bioengineered Stem Cells

Embryogenesis is a sequential process of differential gene expression dictated by the epigenetic environment. Exploiting epigenetic influence on phenotypic outcome, biotechnology platforms are developed for reversal of differentiation to achieve genetic reprogramming of adult sources back to an embryonic state.[43] Such platforms include "therapeutic cloning" and "nuclear reprogramming" that bypass the need for embryo extraction to generate pluripotent stem cell phenotypes from autologous sources.[44,45] Reprogramming of adult stem cells to generate

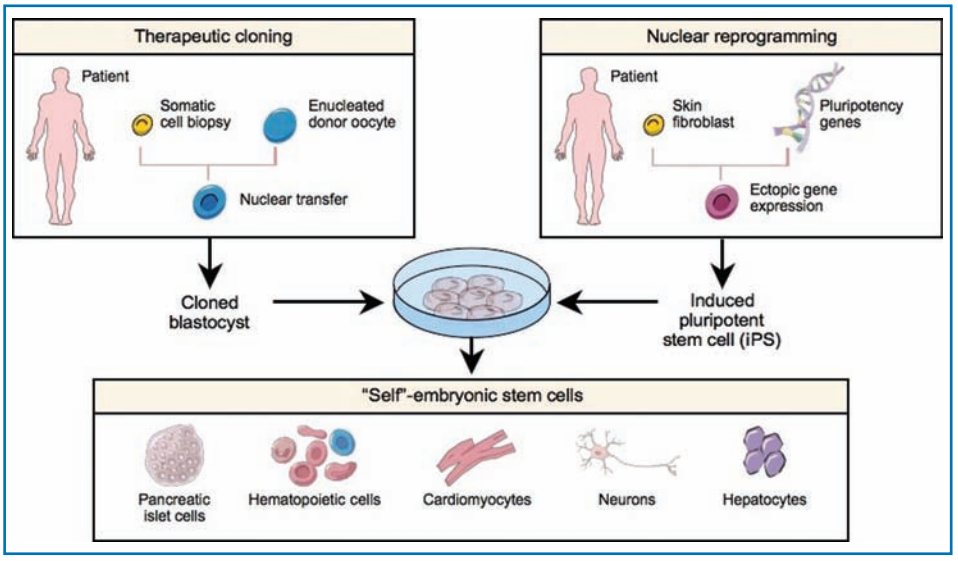

Figure 4. Bioengineered stem cells. Pluripotent stem cells that function like embryonic stem cells can be engineered from adult somatic cells through "therapeutic cloning" or "nuclear reprogramming." Therapeutic cloning combines the nuclear content of a somatic cell obtained from an adult biopsy with cytoplasmic/plasmalemma components from an enucleated donor oocyte. Transfer of the somatic cell nucleus into the remnant of the fertilized egg is performed by micromanipulation. This process results in cloned cells, instructed by the oocyte cytoplasm, that develop into a blastocyst allowing harvest of embryonic stem cells from the inner cell mass (ICM). Alternatively, nuclear reprogramming of fibroblast cells from adults can be achieved through transient ectopic expression of four genes (*OCT4* and *SOX2* with either *NANOG* and *LIN28* or *KLF4* and *c-Myc*) to produce pluripotent cells with embryonic stem cell features, called induced pluripotent stem (iPS) cells. The resulting bioengineered stem cells obtained from both approaches are bona fide pluripotent cells demonstrated by their ability to independently produce an entire organism from embryonic to adult stages of development. Importantly, the tissues derived from these engineered stem cells are genetically similar to the original somatic cell biopsy. This technology produces autologous, embryonic-like stem cells that may enable individualized cell-based therapy.

customized embryonic-like stem cells offers the future for patient-specific regenerative therapies.[46–48]

Nuclear reprogramming of adults cells, namely skin fibroblasts, through ectopic introduction of a small number of pluripotency-associated transcription factors is an alternative approach to induce an embryonic stem cell-like phenotype (*Figure 4*).[49–51] In the mouse, such an approach has yielded induced pluripotent stem (iPS) cells sufficient for *de novo* embryogenesis and germline transmission.[52,53] In humans, transcription factors associated with the pluripotency state were ranked through bioinformatic prioritization and further screened for reprogramming effectiveness through combinatorial viral delivery.[54] Transcription factor sets Oct4, Sox2, c-Myc, and Klf4 or alternatively Oct4, Sox2, Nanog, and Lin28, are sufficient to reprogram human somatic cells to pluripotent stem cells that exhibit the essential characteristics of ESC, including maintenance of the developmental potential to differentiate into advanced derivatives of all three germ layers.[55,56] iPS cell lines should largely eliminate the concern of immune rejection with the ability to derive patient-specific progenitor cells.[48,57] Moreover, iPS-based technology will facilitate the production of cell line panels that closely reflect the genetic diversity of a population enabling the discovery, development, and validation of therapies tailored for each individual.[45] Alleviating technical limitations, including mutation through viral integration,[58] incorporation of oncogenic genes,[59] and establishing reliable patient-specific differentiation protocols,[46,47] are significant advancements that will facilitate clinical translation.

Somatic cell nuclear transfer (SCNT) allows transacting factors present within the mammalian oocytes, conserved across species, to reprogram somatic cell nuclei to an undifferentiated state.[60,61] Therapeutic cloning refers to SCNT in which the nuclear content of a somatic cell from an individual is transferred into an enucleated donor egg to derive blastocysts that contain pluripotent embryonic-like stem cells (*Figure 4*). In this way, SCNT has produced cloned ESCs from multiple mammalian somatic cell biopsies.[62,63] The pluripotency of derived cells has been confirmed through germline transmission and reproductive cloning. However, due to technological limitations cloned human blastocysts have only recently been achieved although in low efficiency,[64] and successful isolation of ESCs from the inner cell mass has yet to be demonstrated with human protocols.

Prospects

Regenerative medicine, founded on the emerging discoveries in stem cell biology, has begun to define the scope of future clinical practice.[65] Regenerative medicine and stem cell biology cross all medical disciplines and provide a universal paradigm of curative goals based on scientific discovery and clinical translation.[66] The full potential of stem cell biology remains unrealized but will continue to require a focused integration of multidisciplinary expertise to form a dedicated and sustained community of practice capable to implement regenerative therapies. Building on the foundation of transplant medicine, regenerative medicine technology is indeed poised to rapidly expand and as a result applications to treat new diseases at earlier stages will provide safer and more effective outcomes. Interventions will range from conception to senescence for both acquired or inherited diseases.[67,68] With significant optimism and hope for successful regenerative medicine applications, unrealistic expectations may offer the most threatening obstacle for the long-term support and progress in this emerging field of therapy. Individualized treatment algorithms to match patients with the most appropriate cell-based intervention according to their inherent reparative deficiencies will facilitate the advancement of personalized solutions.[69,70] In this way, the "stem cell load" for each individual patient will serve as an "index for regenerative potential" useful for prediction, diagnosis, prognosis, and targeting of safe and effective therapies at the earliest stage of disease in the new era of regenerative medicine.

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