Introduction and Basic Concepts in Stem Cell Research and Therapy: The Facts and the Hype

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Contents

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List of Abbreviations

(ACI)	Autologous Chondrocyte Implantation
(ADSCs)	Adipose-derived stem cells
(ALL)	Acute Lymphoblastic Leukemia
(AMD)	Age-related Macular Degeneration
(AML)	Acute Myeloid Leukemia
(BM)	Bone Marrow
(BM-HSCs)	Bone Marrow Hematopoietic Stem Cells
(BM-MSCs)	Bone Marrow Mesenchymal Stem Cells
(CAR)	Chimeric Antigen Receptor
(CBT)	Cord Blood Transplantation
(CFU-F)	Colony Forming-Unit Fibroblast
(CLL)	Chronic Lymphoblastic Leukemia
(CLP)	Common Lymphoid Progenitor
(CML)	Chronic Myeloid Leukemia
(DLI)	Donor Leukocyte Infusion
(DM)	Diabetes Mellitus
(DMT1)	Type 1 Diabetes Mellitus
(DMT2)	Type 2 Diabetes Mellitus
(ECM)	Extracellular Matrix
(ESCs)	Embryonic stem cells
(FTSG)	Full-thickness Skin Graft
(G-CSF)	Granulocyte Colony-stimulating Factor
(GvHD)	Graft versus Host Disease
(GVL)	Graft Versus Leukemia
(HSCs)	Hematopoietic Stem Cells
(HSCT)	Hematopoietic Stem Cell Transplantation
(HSPCs)	Hematopoietic Stem/Progenitor Cells
(iPSCs)	Induced Pluripotent Stem Cells
(ISSCR)	International Society for Stem Cell Research
(MS)	Multiple Sclerosis
(MSCs)	Mesenchymal Stem Cells
(NSCs)	Neural Stem Cells
(OA)	Osteoarthritis
(PB)	Peripheral Blood
(PD)	Parkinson's Disease
(PRP)	Platelet-rich Plasma
(RIC)	Reduced-intensity Conditioning
(RPE)	Retinal Pigment Epithelial
(SCNT)	Somatic Cell Nuclear Transfer

(STSG) Split-thickness Skin Graft
(UCB) Umbilical Cord Blood
(UC-HSCs) Umbilical Cord Hematopoietic Stem Cells
(UC-MSCs) Umbilical Cord Mesenchymal Stem Cells

What You Will Learn in This Chapter

This chapter provides the introduction and overview of stem cells, their definition, origin, and applications. It illustrates the unique properties of stem cells, such as potency, multilineage differentiation potential, self-renewal, and resistance to senescence and apoptosis. It provides a brief description of stem cell research, and its current applications in cell therapy, bone marrow transplantation, tissue engineering and its modern and diverse applications. These will cover approved human stem cell products, and therapies based on cells or their derivatives. Finally, the chapter will cover the gap between research and clinical applications, and concludes with the facts, hope, and hype in stem cell research and development.

1.1 What Is a Stem Cell?

A stem cell is an unspecialized and undifferentiated cell that has a remarkable capacity for self-renewal and the ability to undergo prolonged periods of cell division, both in vitro and in vivo. Stem cells are also capable of asymmetrical division into two non-identical daughter cells with distinctive and different fates. Among the earliest evidence of the existence of stem cells were the breakthrough studies conducted in the early 1960s, when the radiation physicist, James Till, joined with the hematologist, Ernest McCulloch, to study the effects of radiotherapy on hematological cancers in the bone marrow. Among their findings, Till and McCulloch identified a self-renewing population of hematopoietic cells originating in the bone marrow that were capable of generating all blood cell lineages; they named these progenitors "stem cells" [1–3].

Unlike other types of cells, stem cells have the capacity to differentiate into various specialized cells and cell lineages under defined physiological, pathological, and/or experimental conditions. The regenerative capacities are high among younger individuals; aging is associated with lower regenerative potential [4–6]. Moreover, in a mature organism, some organs, such as the blood and intestinal epithelium, maintain a higher rate of regeneration throughout life, whereas other organs, including the heart and pancreas, have limited potential for repair [7]. Stem cells can be classified based on their differentiation capacity into totipotent, pluripotent, multipotent, oligopotent, and unipotent cells, as shown in Fig. 1.1. Totipotent stem cells exhibit the highest capacity for differentiation of any cell in an entire organism, the notable example of this phenomenon is the zygote (i.e., a fertilized egg) which has the capacity to give rise to all embryonic and extraembryonic

structures [8, 9]. Pluripotent stem cells, such as embryonic stem cells (ESCs), are somewhat less potent and are capable of generating embryonic tissues only (i.e., the three germ layers, mesoderm, endoderm, and ectoderm [10]). Lineage specific multipotent stem cells such as mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) have a more restricted capacity for differentiation and give rise to their specific tissues and cell types [11]. Oligopotent stem cells are even more restricted but maintain the capacity to differentiate into specific cells within specific tissues. A good example of an oligopotent stem cell is the common lymphoid progenitor (CLP), which can give rise to T lymphocytes, B lymphocytes and natural killer cells [12, 13]. Unipotent stem cells are the most restricted, as they are capable of generating cells of a single lineage; examples of unipotent stem cells include epidermal stem cells of the skin [14, 15], myogenic precursors [16], and spermatogonial stem cells [17].

It is generally understood that the capacities for self-renewal and differentiation diminish as cells become more specialized. However, this dogma was recently challenged by the successful reprogramming of fully differentiated somatic cells into a pluripotent-like state in the form of somatic cell nuclear transfer (SCNT) [18] and likewise via the induction of pluripotent stem cells (iPSCs), first described in 2006 [19].

1.2 Origin and Types of Stem Cells

Stem cells are classified as embryonic or adult stem cells based on their source of origin (as shown in Fig. 1.1). Tissues associated with pregnancy, including the placenta, amniotic fluid, umbilical cord, and Wharton's jelly, among others, are all rich in stem cells. Likewise, iPSCs are cells produced by the direct reprogramming of somatic cells into pluripotent stem cells. A comparison of the properties of embryonic, adult, and iPSCs is presented in Table 1.1.

1.2.1 Embryonic Stem Cells (ESCs)

ESCs can be collected from the inner cell mass of pre-implantation embryos 3–5 days following fertilization. ESCs are pluripotent cells that have the capability to divide for extended periods of time and to differentiate into cells of each of the three germ layers [10, 20]. This robust differentiation potential qualifies ESCs as the best-known source of cells that can be used to generate fully differentiated cells for cell therapy applications [21, 22]. Ethical concerns related to the destruction of human embryos have hampered the full application of ESCs, which are isolated from spare/discarded embryos that were generated to support in vitro fertilization (IVF) procedures and not from healthy in utero-implanted ones [23–25].

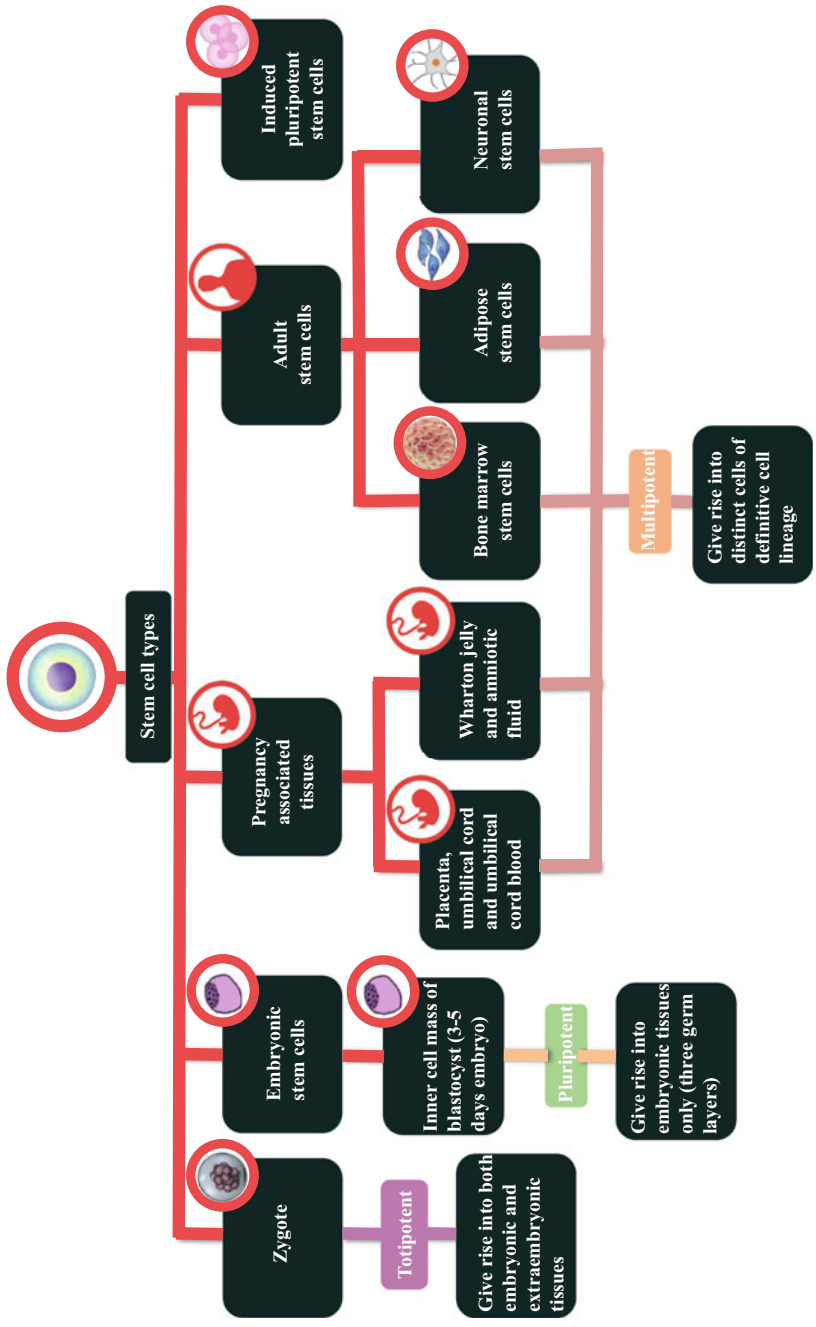

Fig. 1.1 The origins and types of stem cells

Table 1.1 General comparison between embryonic stem cells, adult stem cells, and iPSCs

Cell type	Embryonic stem cells	Adult stem cells	iPSCs
Origin	Pluripotent cells derived from the inner cell mass of the blastocysts [10, 43]	Multipotent cells derived from adult tissues [37, 44–46]	Somatic cells reprogrammed into embryonic-like pluripotent stem cells [19, 47]
Self-renewal capacity	High [10, 43]	Limited [37, 44–46]	High [19, 47]
Potency	Pluripotent [10, 43]	Multipotent [37, 44–46]	Pluripotent [19, 47]
Differentiation	Can differentiate into cells of each of the three germ layers [10, 43]	Restricted lineage differentiation [37, 44–46]	Can differentiate into cells of each of the three germ lineages [19, 47]
Surface markers	Pluripotency markers (OCT4, SOX2, NANOG, SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81 [10, 43, 48]	Specific markers of adult tissue-derived stem cells. For example, MSCs express CD90, CD73, and CD105 along with a negative expression for the hematopoietic markers CD45, CD3, CD19, CD11, CD79α, and human leucocyte antigen-DR (HLA-DR) [46, 49]	Pluripotency markers (OCT4, SOX2, NANOG, SSEA-4, and KLF4 [19, 47, 50]
Spontaneous oncogenic transformation	Present [10, 43]	Absent [37, 44–46]	Present [19, 47]
Immune response	Strong [51, 52]	Strong for allogeneic, but not for autologous cells [53–55]	Strong, but can be minimized for autologous cells [56]
Ethical concerns	Yes [24, 51]	No [57]	Minimal [58]

1.2.2 Adult Stem Cells

Somatic or adult stem cells are rare populations of undifferentiated cells that are found among their differentiated counterparts throughout the adult body. These cells contribute to tissue homeostasis, as they serve as a source of raw material for repair and/or replacement of injured or dead cells [5]. Adult stem cells have only a limited range of differentiation potential when compared with ESCs. Examples of adult stem cells include the following:

- **Mesenchymal Stem Cells (MSCs)**

MSCs are adherent fibroblast-like cells when cultured in vitro. They were first isolated from the bone marrow [26, 27], where they are most abundant. They produce colony forming-unit fibroblast (CFU-F), when cultured in vitro and are distinguished by the capacity to differentiate into osteocytes, chondrocytes, and adipocytes. There are numerous sources of MSCs including bone marrow [28], adipose tissue [29], dental pulp [30], and synovial membranes [31].

- **Hematopoietic Stem Cells (HSCs)**

HSCs have been isolated from the bone marrow; they have the capacity for self-renewal as well as the ability to differentiate into all blood cell lineages [3]. They are widely used clinically in HSC transplantaion for treating various blood disorders and malignancies.

- **Neural Stem Cells (NSCs)**

NSCs are found in the central nervous system; they have the potential to differentiate into both neuronal and non-neuronal glial cells [32]. As such, they have been used clinically in efforts to repair injuries sustained by the nervous system [33, 34]. Currently, the use of NSCs for treating neurodegenerative diseases is under investigation [35].

1.2.3 Other Stem Cells

The discovery of stem cells in the human umbilical cord blood (UCB) paved a new and useful source of progenitors; notably umbilical cord blood hematopiotic stem cells (UCB-HSCs) have become a viable source of autologous bone marrow stem cells. UCB-HSCs are capable of differentiating into multiple hematopoietic lineages, in addition to their capacity for long-term self-renewal [36, 37]. Clinically, UCB stem cells have been employed successfully as HSC transplants in 1988 [38]. As such, parents in some countries now routinely bank the UCB of newborns so as to have a source of HSCs in the advent of any childhood hematological disorders or malignancies. Likewise, as noted earlier, MSCs have been identified in extraembryonic tissues, including Wharton's jelly [39], amniotic membrane and placenta [39, 40], and amniotic fluid [41].

1.2.4 Induced Pluripotent Stem Cells (iPSCs)

iPSCs are generated in vitro in an effort to imitate the potential of ESCs by effectively reversing the differentiation of somatic cells (e.g., skin fibroblasts) in order to become pluripotent [19, 42]. The discovery of iPSCs was driven at least in part by the need to identify ESC-like pluripotent stem cells for clinical use which could be generated without

raising strong ethical concerns. Many ongoing efforts are aimed at improving current reprogramming approaches so as to enhance the current clinical applicability of iPSCs.

1.3 Stem Cell Therapies: The Present and the Future

The remarkable potential of stem cells, including their capacities for self-renewal and differentiation, has led to their use in numerous clinical applications, including cell-based therapies [59], drug discovery [60], and tissue engineering [61]. The ultimate goal of stem cell-based therapies is to treat, repair, or replace diseased tissues or organs with ones that are new, healthy, and functional [62, 63]; numerous applications of this type are presented in Fig. 1.2. Therefore, stem cells are currently featured in several thousand ongoing clinical trials focused on disease treatment.

Most of these protocols focus on the use of stem cells for treating hematological disorders, including myeloid leukemia; lymphoma; sickle cell anemia; immune deficiencies; β-thalassemia [64–67]; wound healing and skin injuries [68]; neurological disorders, such as Parkinson's diseases and spinal cord injury [69, 70]; autoimmune disorders, such as multiple sclerosis, rheumatoid arthritis, Crohn's disease, and type-1 diabetes [71–74]; and cardiac diseases, including ischemic heart disease [75]. Promising trials, which focus on the use of stem cells to treat ocular disorders, including macular degeneration and retinitis pigmentosa [76, 77], and bone diseases, including osteosarcoma,

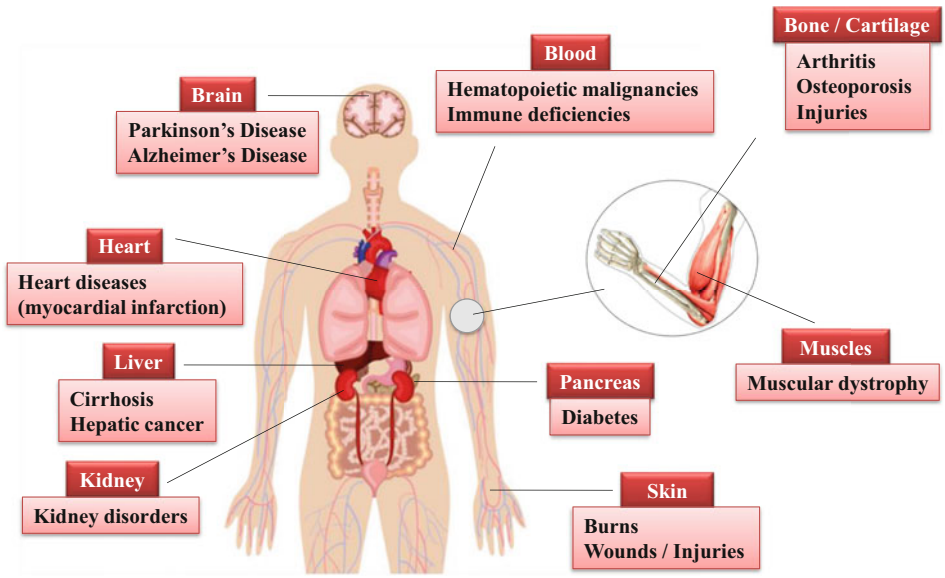

Fig. 1.2 Stem cell therapy for chronic diseases

osteoporosis, and osteoarthritis, are also in progress [78, 79]. So far, only a handful of the U.S. Food and Drug Administration (FDA) approved stem cell products are available for clinical use, including allogeneic cord blood hematopoietic stem/progenitor cells for treating hematological and immunological disorders (https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products). Currently approved stem cell-based therapies are listed in Table 1.2.

Table 1.2 Approved human stem cell-based products

Approved products	Used stem cell type	Indications	Approval status	Approved by
ALLOCORD	Allogeneic cord blood hematopoietic progenitor cell	Used in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution of patients with inherited or acquired disorders of the hematopoietic system or as a result of myeloablative treatment.	Approved	Office of Tissues and Advanced Therapies of the FDA (USA)
CLEVECORD				
DUCORD				
HEMACORD				
HPC, Cord Blood				
HPC, Cord Blood—MD Anderson Cord Blood Bank				
HPC, Cord Blood—Life South				
HPC, Cord Blood—Blood works				
HOLOCLAR	Ex vivo expanded autologous human corneal epithelial cells containing stem cells	Treatment of adult patients with moderate to severe unilateral or bilateral limbal stem cell deficiency due to physical or chemical ocular burns.	Conditional Approval	European Medicines Agency (EU)
ZYNTEGLO	Autologous $CD34^+$ hematopoietic stem cells transduced with lentiviral vector encoding the human $beta^{A\text{-}T87Q}$-globin gene	Treatment of beta thalassemia.	Conditional Approval	

1.3.1 Routine Stem Cell Therapy for Hematopoietic Disorders

1.3.1.1 Hematological Malignancies

Transplantation of unmodified or genetically modified HSCs derived from different sources offers a promising approach to the reconstitution or replacement of diseased cells. Cell therapies for hematological disorders, such as hemoglobinopathies (e.g., sickle cell anemia) and blood malignancies (e.g., leukemia and lymphoma), have undergone substantial development over the past few decades, as in the examples discussed below [80].

Leukemia

Leukemias are a group of white blood cell malignancies classified by the World Health Organization (WHO) based on genetics, morphology, immunophenotype, and clinical features [81, 82]. Interestingly, one of the earliest known cases of leukemia was identified based on the findings from an Egyptian skeleton in dating back to 2160–2000 BCE [83]. Leukemias are classified into several major subtypes, including acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), and chronic lymphoblastic leukemia (CLL) [84]. Chemotherapy was an initially effective treatment for childhood ALL when first attempted in 1948; unfortunately, disease typically relapsed ultimately leading to death [85, 86]. Currently, the standard treatment includes combination chemotherapy to destroy the defective hematopoietic system followed by hematopoietic stem cell transplantation (HSCT) [87, 88]. This approach is particularly indicated for recurrent disease, and can be introduced shortly after first-line treatment with chemotherapy [89, 90]. HSCs can be derived from the bone marrow (BM), umbilical cord blood (UCB), or peripheral blood (PB) [91]. The first successful allogeneic human bone marrow transplantation (BMT) performed in patients with leukemia following optimized radiation and chemotherapy doses resulted in a Nobel Prize in Medicine for Dr. E. Donnall in 1990 [92]. However, histocompatibility mismatching and graft rejection resulted in high relapse rates; as such, the disease relapsed and the success rate was low [93]. Among the efforts made to improve these outcomes, donor leukocyte infusions (DLI) were introduced, by providing immune cells pre-collected from the anticipated HSC donor following myeloablation in patients undergoing leukemia treatment; the goal was to establish donor chimerism and thereby preventing graft rejection [94]. Although, DLI was effective in managing disease relapse, it was related to the development of graft *versus* host disease (GvHD) in treated patients, resulting from the activity of effector donor T-cells [95]. Reduced-intensity conditioning (RIC) was also applied in an effort to control graft *versus* host disease (GvHD), while enhancing the graft *versus* leukemia effect (GVL), thereby maintaining engraftment and eradicating malignancy [96]. The use of less aggressive RIC and non-myeloablative conditioning reduces the overall toxicity and mortality associated with conditioning prior to transplantation, especially in older patients [96].

The relatively recent inclusion of UCB as a source for HSCs overcame the challenges associated with an attempt to locate an HLA-matched allogeneic donor [97]. UCB cells

were also less immunogenic and also easy to collect; UCB cells cryopreserved for decades still support the efficient recovery of HSCs [98]. However, UCB maintains comparatively fewer HSCs with respect to adult weight; as such, two bags of cord blood are typically required in order to obtain a sufficient yield of HSCs for transplantation into a single patient [99–101]. Nonetheless, a long-term follow-up of the Eurocord–European Group for Blood and Marrow Transplantation study revealed encouraging results. The study evaluated the outcome of UCB transplantation for 147 children, among whom 74% had been diagnosed with acute leukemia. In these patients, the cumulative incidence of neutrophil recovery was 90% at 2 years post-transplantation, the incidences of acute and chronic GvHD were reported to be 12% and 10%, respectively. At 5 years post-transplantation, the cumulative incidences of relapse and non-relapse mortality were 47% and 9%, respectively; the probability of disease-free survival was 44%. These results stand in strong support of UCB banking and the use of cord blood units to facilitate HLA-identical cord blood transplantation (CBT) [102].

PB-HSCs can be collected by noninvasive means; this provides a safe procedure for both the donor and recipient who can then undergo more rapid engraftment [103]. Administration of recombinant granulocyte colony-stimulating factor (G-CSF) stimulates the release of endogenous HSCs from the BM and into the blood. Currently, about 80% of all allogeneic transplantations are performed using stem cells derived from the PB of adult patients [104]. Similarly, recent developments in targeted therapy approaches have resulted in improved outcomes and can eliminate the negative sequelae associated with indiscriminate cytotoxic myeloablation. Genetically modified T-cells that express antigen-specific chimeric antigen receptor (CAR) will target leukemic cells while sparing those that are otherwise normal [105]. The FDA has approved the use of autologous genetically modified CD19-lymphocyte cells (CAR T-cells) for the treatment of relapsed ALL and diffuse large B-cell lymphoma [106].

Sickle Cell Anemia

In addition to traditional HSC transplantation, it is now possible to manipulate the diseased cells by removal, addition, or alteration of specific DNA sequences in order to correct defective or mutated genes. High efficiency and precise genetic manipulation or gene editing of the human genome has recently become possible with the use of the method known as clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 [107]; this procedure is outlined in Fig. 1.3. CRISPR/Cas9 was used to restore the normal blood cell phenotype by repairing $CD34^+$ hematopoietic stem/progenitor cells (HSPCs) from patients diagnosed with sickle cell anemia, a disorder that typically results from a single nucleotide substitution within a β-globin gene [108]. The gene-edited HSPCs were transplanted back into the patient's BM to function as a source of healthy autologous red blood progenitors; using this method the disease undergoes genetic correction, and graft rejection is evaded [108].

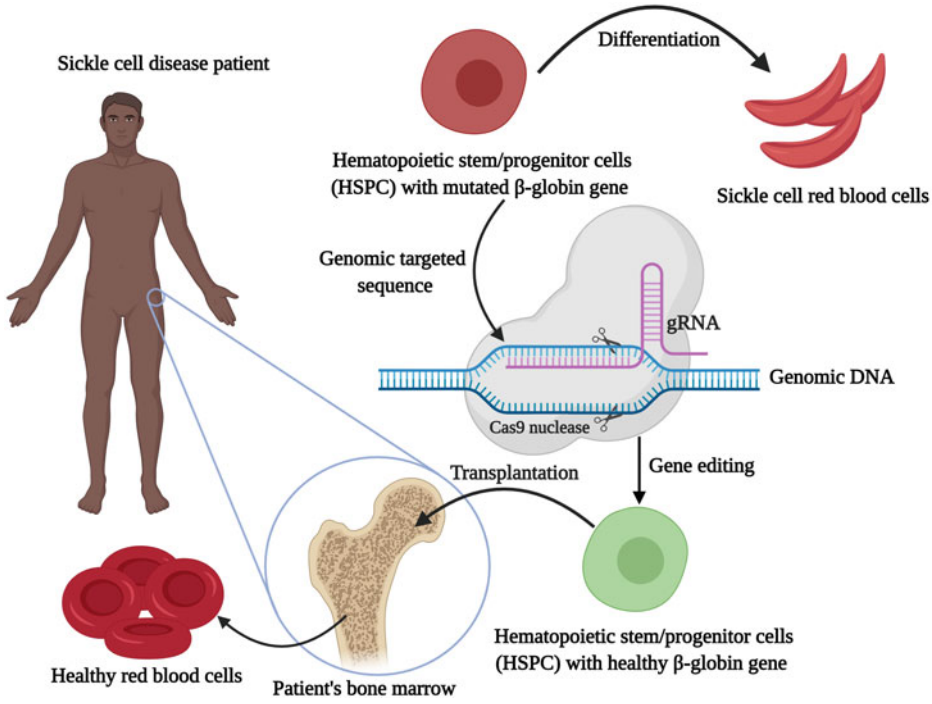

Fig. 1.3 Illustration of CRISPR/Cas9 gene editing

1.3.2 Stem Cell Therapy in Clinical Trials

1.3.2.1 Skin Injuries and Wound Healing

Skin is the largest organ in the body and a major part of the integumentary system that covers and protects the human body [109]. Physical, chemical, and biological factors can all disrupt skin integrity. Depending on the depth of injury, skin wounds can be epidermal, or they can involve either partial or full skin thickness [110]. The natural healing mechanisms are compromised by third- and fourth-degree burn injuries; this presents a significant challenge for both the surgeons and patients. Over the past century, the gold standard for treating burns has been grafting of healthy skin. Skin grafting can include split-thickness skin graft (STSG) and full-thickness skin grafts (FTSG) [111, 112]. Skin grafting involves the transfer of healthy skin (autograft or allograft) comprised of the epidermis and a portion of the dermis to the site of injury; problems arise when there is not enough healthy skin, a failure to treat deep wounds, a poor cosmetic outcome, and limited strength of grafted skin when compared with the original skin at the affected site [113]. Skin engineering thus represents an attractive alternative. Autologous keratinocytes or fibroblasts are cultured on a scaffold, in some cases, a scaffold alone is implanted into the wound to improve healing [114]. This technique results in the regeneration of both the epidermal and

dermal layers; however, this method did not facilitate the regeneration of skin appendages, including hair, nails and skin glands. Of note, traditional skin grafting also failed to regenerate skin appendages; however, pigmented melanocytes and neural and vascular tissues were recovered using this method, an outcome that was not achieved using the engineered skin [114]. Skin replacements can be generated using cellular or acellular scaffolds; based on the composition of the skin-substitute [115]. Acellular skin-substitutes are biodegradable scaffolds (e.g., collagen, elastin, and silicon, among others) that facilitate wound healing by recruiting fibrocytes and vascular cells in vivo and by inhibiting granulation and scar formation. The most common acellular skin-substitutes currently approved by the FDA and undergoing review in clinical trials include Integra® [116], Alloderm™ [117], and NovoSorb™ BTM (Biodegradable Temporizing Matrix) [118]. Cellular skin-substitutes that contain epidermal cell sheets include Dermagraft® and Apligraf®; these products were approved by the FDA for the treatment of diabetic foot ulcer [118, 119]. ReCell® is an FDA-approved commercial cell spray device that provides autologous keratinocytes designed to heal second-degree burns. ReCell® works by facilitating enzymatic digestion of the patient's healthy skin in order to harvest keratinocytes, which are then sprayed over the wound [120, 121]. Commercially available skin-substitutes are still far from perfect. The cells frequently fail to integrate; show poor vascularization, weak mechanical integrity, and scar formation; and are subjected to immune-mediated rejection [109]. Indeed, there are no completely functional skin-substitutes available at this time; of particular note, there is a great need for a functional skin-substitute that can undergo rapid vascularization. Recent advances in stem cell therapy, nanotechnology, tissue engineering, and microfluidics paved the way for improved skin tissue engineering focused on deep wound healing [122]. Bioscaffolds for skin engineering must all be biocompatible, nontoxic, non-immunogenic, biodegradable, and sufficiently porous so that free exchange of gases and nutrients can occur through a neo-vascularized functional skin-substitute [123]. The cell source for the engineered skin also has a significant impact on the outcome. For example, ESCs can be differentiated into both keratinocytes [124] and fibroblasts [125], but direct clinical applications of these cells are hampered by instability and concerns with respect to the functionality of the resultant tissues. Adipose-derived stem cells (ADSCs) can also differentiate into keratinocytes, fibroblasts, and other skin components; ADSCs also produce extracellular matrix (ECM) which is rich in growth factors and cytokines that enhance healing [126–128]. The ADSC secretome contains vascular endothelial growth factor (VEGF), growth differentiation factor (GDF-11), and transforming growth factor (TGF-β); all of these act on macrophages, fibroblasts, and endothelial cells and lead to limiting the immune responses, enhancing cell proliferation, and promoting angiogenesis at the transplantation site [129]. Clinical applications of autologous ADSCs are still under investigation for healing diabetic foot ulcers (NCT02092870, see https://www.clinicaltrials.gov) [130]. Furthermore, methods used to generate three-dimensional skin grafts using iPSC-derived keratinocytes and fibroblasts remain promising [131].

1.3.2.2 Osteoarthritis

Osteoarthritis (OA) is a chronic degenerative disease characterized by deterioration of joint articular cartilages; this results in exposed subcondylar bones and leads to friction, pain, and synovitis [132]. Globally, OA is currently estimated as the 11th highest contributor to adult disability; this results largely from pain, stiffness, and impaired mobility due to disease affecting the knees, feet, hands, and spine joints [133]. Non-surgical approaches for treating OA include intra-articular injections of corticosteroids, hyaluronic acid "viscosupplementation," or autologous platelet-rich plasma into the deteriorating joints [134–136]. These approaches are designed to alleviate pain, but they do not treat the underlying cause of mechanisms associated with OA [137]. Joint surgery for OA varies from whole knee replacement (arthroplasty) to minimally invasive arthroscopic techniques such as microfracture or microdrilling [138–140]. The aforementioned arthroscopic techniques involve the generation of multiple small fractures within the affected joint, promoting the recruitment of progenitor cells from the underlying BM which then undergo differentiation into chondrocytes [139]. The drawbacks of these approaches include the formation of an inferior form of cartilage that lacks mechanical durability [138].

Alternative cell-based approaches have been applied, including osteochondral transplantation and soft tissue grafting [141]. Among the problems associated with these approaches, outcomes have included poor grafting and integration, calcification of the grafts, and limited number of available donor tissues [142, 143]. Accordingly, more effort has been directed toward autologous/allogeneic chondrocyte implantation (ACI) [144]. Currently, there are numerous phase III clinical trials involving ACI that include the expansion of autologous or allogeneic chondrocytes, followed by grafting into the deformed lesion [145]. As an example, a phase III clinical product that is now commercialized with the brand name Chondrosphere® utilizes scaffold-free spheroids of chondrocytes obtained from autologous articular cartilage that are introduced for use to treat cartilage defects associated with hip injuries (NCT01222559) [146]. The challenges currently encountered include increased susceptibility of the donor to OA after tissue sampling in normal joints and an overall insufficient number of harvested chondrocytes. Likewise, expanded chondrocytes may undergo dedifferentiation and lose their ability to generate cartilage matrix [147].

MSCs have also emerged as a promising source of cells for this application owing to their robust capacity for expansion and chondrogenic differentiation [148, 149]. In addition, MSCs secrete a variety of cytokines and growth factors with anti-inflammatory effects [150]; these cytokines may function to counteract the inflammatory processes associated with OA. Autologous bone marrow-derived MSCs have been used to repair full-thickness cartilage defects in two cases [151]. In this study, BM was aspirated from the iliac crests and cultured until adherent MSCs had undergone several expansion passages. Cultured MSCs were then collected, embedded in a collagen-gel scaffold, and transplanted onto the surface of the defective articular in the knee joint. Symptoms were relieved at 6 months, and both male and female patients were satisfied with the outcomes during the 4 years following transplantation [151]. MSCs derived from the umbilical cord, placenta,

Wharton's jelly or amniotic membrane all have shown promise with respect to novel treatments for patients diagnosed with OA [152–154]. In particular, UC-MSCs exhibited higher proliferative, clonogenic, anti-inflammatory, and chondrogenic potential compared with MSCs from maternal-derived decidua or BM [155]. CARTISTEM® is a commercialized product that utilizes UC-MSCs for the treatment of cartilage deterioration in patients with OA; it is currently approved for a phase III clinical trial with the goals of evaluating safety and expanding its indications for use (NCT01041001, NCT01626677). Recently, phase II clinical trials have been initiated to assess the role of ADSCs for the treatment of patients with OA (NCT02838069) [78].

1.3.3 From Bench to Bedside

1.3.3.1 Diabetes Mellitus (DM)

Diabetes mellitus (DM) is a chronic inflammatory metabolic disorder that results in sustained hyperglycemia due to defects in insulin production (Type I), insulin utilization (Type II), or a combination of both [156]. Type I DM (T1DM) is an autoimmune disease, wherein activated immune cells attack insulin-secreting β-cells in the pancreas, resulting in insulin deficiency [157]; contrarily, type II DM (T2DM) is characterized as a chronic inflammation state that ultimately leads to insulin resistance, reduced insulin secretion, β-cells exhaustion, and apoptosis [158–160]. Untreated DM leads to severe complications that can be life-threatening and have significant impact on numerous major organs including the kidneys [161], heart [162, 163], eyes [164, 165], and nervous system [166].

Patients with diabetes attempt to regulate their blood glucose levels and to maintain values at or near normal limits with dietary control [167], hypoglycemic drugs [168], and lifestyle changes [169]. However, these traditional methods often fail to maintain normoglycemia in the long run [170]. Islet transplantation (also known as Edmonton protocol) was developed in 1999 to provide more β-cells and thus increase insulin production for patients diagnosed with T1DM [171–173]. However, the use of this approach was limited due to the risks associated with the surgical procedure [174], the need for long-term immunosuppressive therapy [175], a shortage of organ donors [176], and only limited impact with respect to achieving insulin independence [177].

Stem cell-based therapy provides a new approach for the management and treatment of DM. First, this approach can create a virtually unlimited supply of insulin-producing cells [178–181]; other applications focus on restoring β-cell function [182], modifying immune dysregulations, and reversing the associated metabolic complications [183]. Pluripotent ESCs were successfully differentiated into β-cells in vitro [184, 185]; results in vivo revealed that insulin production and normal blood glucose level were sustained at 3 months post-transplantation [186]. Despite these promising results, there are few clinical trials addressing this approach, and there is currently no reliable information on its safety or efficacy (https://www.clinicaltrials.gov/).

Considering the different embryological origins of MSCs and pancreas, MSCs showed variable responses to the efforts made toward differentiating them into pancreatic β-cells. For instance, BM-MSCs failed to adopt functional characteristics of β-cells when cultured in vitro [187]; contrarily, ADSCs revealed some genetic and morphological similarities to pancreatic cells [188, 189]. However, MSC-mediated immunomodulation and inhibition of autoimmune progression may be achieved by educating autoreactive T lymphocytes, an approach in which the autoreactive T-cells are being regulated to be less reactive to the patient's own islet cells, thereby reducing the extent of β-cell destruction in patients diagnosed with T1DM [181, 190, 191]. Moreover, for T2DM patients, the transplantation of autologous MSCs would reduce the associated inflammatory reactions and promote pancreatic healing [181, 192, 193]. Several clinical trials (NCT03343782, NCT01068951 and NCT01759823) demonstrated that autologous BM-MSC transplantation was a promising approach, as it coupled long-term efficacy and safety vis à vis the diabetic microenvironment [194–196]. Results from a limited number of trials for T1DM patients revealed improved clinical outcomes in patients treated with UC-MSCs than in those treated with BM-MSCs, although BM-HSCs were more effective than UCB-HSCs [197]. Despite the fact that stem cell therapy may ultimately overcome many of the well-known limitations of traditional DM therapy, more clinical trials are still required. At this time, short follow-up periods, small number of patients, missing control groups, and lack of standardization of the transplantation protocols were major setbacks for some of the clinical trials [196, 198].

1.3.3.2 Multiple Sclerosis (MS)

Multiple sclerosis (MS) is a chronic, autoimmune, inflammatory, and neurodegenerative disorder of the central nervous system [199]. MS is characterized by demyelination with axonal loss and long-term progressive disability due to disease exacerbation with the inflammatory microenvironment that enhances local oxidative stress and hypoxia [200, 201]. Several pharmacological and non-pharmacological therapies are currently approved for the treatment of MS; however, these treatments may only delay disease progression and reduce the severity of its symptoms [202]. Consequently, therapies that promote remyelination of injured axons remain among the challenges.

HSCT has been used to treat MS following high dose chemotherapy for immunosuppression [203]; this modality aims to reboot the immune system and eliminates autoreactive T- and B-cells, thereby facilitating the generation of a new and tolerant immune system [203]. HSCT has since become an alternative option for the treatment of other autoimmune-related diseases as well [204–207]. Despite the improvements observed in some MS patients, the high risk of chemotoxicity and immune deficiency in this patient cohort remains an important drawback to widespread implementation [208, 209].

MSCs have unique immunomodulatory and anti-fibrotic properties [210, 211] and are thus attractive choices for the development of targeted treatments for MS. Autologous BM-MSC transplantation resulted in diminished production of pro-inflammatory cytokines in association with improved vision and movement in patients diagnosed with MS

[212, 213]. In another trial, UC-MSC transplantation resulted in improvements in physical movement with fewer side effects [214]. However, the potential therapeutic effects and mechanism of action of these cells require further investigation.

1.3.3.3 Parkinson's Disease (PD)

PD is the second most prevalent neurodegenerative disease worldwide with an incidence that increases with age [215]. Characterized by gradual death of the dopaminergic neurons in the substantia nigra of the brain, PD leads to motor nerve impairment and reduction in the capacity for voluntary movements [216]. The exact cause of PD remains under investigation, however, the gene encoding α-synuclein (SNCA) was found to be involved with the abnormal accumulation of Lewy bodies inside neurons [217, 218]. There is currently no cure for PD; however, specific drugs are reasonably effective in restoring dopamine concentrations, as well as improving motor neuron function and relieving symptoms characteristic of PD. Nevertheless, these medications are often associated with off-target adverse events in long-term use [219, 220]; this limits their overall efficacy.

Pluripotent stem cells have the capacity to differentiate into dopaminergic neurons in vitro [221–223]. ESCs underwent efficient differentiation into midbrain dopaminergic neurons. When grafted into the striatum, these cells promote motor improvement, improved graft survival, and reduced levels of teratoma formation in mice [224]. A phase I/II clinical trial is currently underway, which aimed to investigate the safety and efficacy of neural precursor cells generated from human ESCs (NCT03119636) [225]. In addition, iPSCs are also promising candidates, in terms of the possible generation of dopaminergic neurons for transplantation to treat PD [226]. A personalized medicine approach revealed that differentiated dopaminergic neurons generated from autologous iPSCs could limit the progression of PD for 18–24 months [227]. A clinical trial designed to evaluate the efficacy of this approach in PD patients is currently ongoing (NCT00874783) [47].

Administration of MSCs that differentiated into dopaminergic neurons resulted in improved movement after transplantation using PD mouse models [228, 229]. Interestingly, MSCs were also found to exert a neuroprotective effect via their capacity to regulate both autophagy and α-SNCA expression, thereby rectifying PD brain-microenvironment [230]. In addition, the introduction of MSC-associated secretory factors and exosomes was associated with outstanding results in PD animal models [231–233]. BM-MSCs are the most commonly used cells in clinical trials; administration of autologous and allogeneic BM-MSC transplantation resulted in improved movement in three of seven patients; another two patients tolerated a reduction in PD drugs following BM-MSC transplantation [234]. No serious health concerns were reported during the 12–36-month trial; these findings encourage further testing of the BM-MSC transplantation in a larger number of patient cases [234]. Recently, administration of UC-MSCs resulted in promising outcomes in experiments conducted using PD animal models [235–237]; two clinical trials exploring both the efficacy and safety of this approach are ongoing (NCT03684122 and NCT03550183).

Administration of NSCs also resulted in positive outcomes with respect to treatment of PD; these cells released neurotrophic factors that enhance neural functions and promote their migration to the site of the lesion, thereby facilitating repair of damaged tissue [238]. One clinical trial (NCT03815071) is currently testing the efficacy of administration of autologous NSCs to patients diagnosed with PD; more trials are required in order to evaluate the long-term efficacy and safety of the use of NSCs under these conditions.

1.3.3.4 Age-related Macular Degeneration (AMD)

Age-related macular degeneration (AMD) is an incurable disease resulting in the gradual loss of vision in one or both eyes [239, 240]. The macula, which is the central part of the retina, contains the photoreceptors (rods and cones) and is essential for central vision, perception of details, and differentiation among colors within a field of vision [241, 242]. Retinal pigment epithelial (RPE) cells are supportive cells that provide nutrition to retinal photoreceptors. In macular degeneration, RPE cells degenerate and fail to support the retina, resulting in the loss of central vision, blurred visual fields, and diminished capacity for color discrimination [240]. Macular degeneration exists in both wet exudative and dry non-exudative forms [239]. The dry type is associated with thinning and death of the RPE cells and is associated with yellow deposits (drusen), whereas the wet type involves the formation of new blood vessels and bleeding beneath the retina [240].

The current treatment for AMD focuses on delaying its progression, via the administration of antioxidants or anti-VEGF for patients diagnosed with dry or wet AMD, respectively [243–246]. While these therapies result in slight improvements in retinal function, they do not restore degenerating RPE cells. As such, preclinical studies have focused on transplantation of retinal progenitor sheets in an effort to replenish RPE cells in the injured area of the eye; this approach has shown promising results by improving vision in mice [247–250].

Recently, the use of pluripotent stem cells for the repair of macular damage gained much attention. ESCs can differentiate in vitro into photoreceptor cells [251] that can then be transplanted into the eyes of an individual diagnosed with AMD; through this method, human ESC-derived RPE cells were injected directly into the injured eye. The results of preliminary studies revealed that this method is safe and that there is little immune rejection of the transplanted cells; the ESC-derived RPE cells were genetically stable, did not generate tumors, and maintained strong differentiation to >99% pure RPE cells (NCT01345006 and NCT01344993) [77, 252]. However, concerns regarding genetic instability and the potential for tumorigenesis when administering pluripotent stem cells for the treatment of AMD were recently addressed [253, 254]; a recent study aimed to validate the safety of ESC-derived RPE cells through genomic analysis [255]. Furthermore, iPSC cell lines were recently differentiated into three-dimensional retinal organoids which may be useful for replacing damaged photoreceptors [256]. Reprogramming of autologous skin fibroblasts into iPSCs, then their differentiation into RPE cells, has also been investigated (Clinical trial UMIN000011929) [257].

Although MSCs were tested repeatedly for their capacity to differentiate into neuronal cells or photoreceptors [258, 259], recent studies revealed that these cells should not be used to treat AMD. Despite the absence of appropriate preclinical studies, some physicians rushed forward and use MSCs in AMD treatment protocols; this unfortunately led to several incidents of complete blindness. As but one example, a 2017 report described the case of a 77-year-old woman who received autologous adipose MSC injections into both eyes, at a clinic in Georgia; she experienced bilateral retinal detachment and complete blindness at 3 months following the procedure [260].

1.4 Stem Cell Therapies: Facts, Hope and Hype

Stem cell therapies are among the most exciting and revolutionary medical advances of the twenty-first century. They are frequently described in the media as a "wonder-cure" or "cure-all." Indeed, clinical applications of stem cells are increasing in number worldwide as its research progresses and matures. It remains important, however, to balance patients' needs and desires with the fact that there are currently no well-established clinical outcomes from any stem cell-based protocol. Unfortunately, several clinicians have undertaken a "rogue" approach by misusing stem cell therapy and providing services to patients that go beyond currently approved applications [261]. Moreover, false marketing and unsubstantiated advertising in almost all media outlets feature unapproved stem cell therapies for conditions ranging from mild cosmetic enhancements to cure for intractable organ failure.

By 2018, more than 430 established enterprises in the USA were promoting numerous variants of stem cell therapy (all types of stem cells for so many diseases) in more than 710 clinics distributed in various states [262]; these numbers indicate a profound increase over those reported only 2 years earlier (i.e., during 2016 [263]). Taking together, these findings indicate an increasing trend toward embracing uncontrolled and unproven stem cell therapies. Moreover, in a study conducted in 2017, researchers found that only 43.6% of a total of 408 funding campaigns focused on stem cell therapy reported true and verifiable information in terms of efficacy, and only 8.8% mentioned the risks associated with their use [264]. Most of these businesses asserted scientific legitimacy by referring to published articles in journals with little or no scientific peer-review, and provided false claims regarding their involvement and relationship with preclinical research conducted at reputable research centers [265].

Warnings are issued constantly by the FDA, the U.S. Centers for Disease Control (CDC), Euro Stem Cell, the International Society for Stem Cell Research (ISSCR) as well as other international stem cell consortiums regarding the premature use of stem cells in clinical sittings. These cautions are fully justifiable, since claims of efficacy and safety of several uncontrolled and improperly identified stem cell therapies are portrayed with optimistic messages; that often ignore the associated risks and/or potential for adverse reactions [266, 267]. As such, there is a compelling need to increase patients' awareness of

what therapies are actually clinically approved as opposed to what is currently advertised inappropriately.

Some forms of stem cell therapy, particularly the use of HSCs for hematopoietic disorders, have been the subject of extensive research, are clinically proven, and have been established as routine standard of care. The skin stem cells used for treating severe burns have shown considerable promise as well as treating immune deficiencies and solid cancers. However, other modalities featuring stem cells are still under experimental investigation and have not yet been approved for clinical use.

Validated clinical trials are required in order to provide the utmost guarantee of safety and efficacy prior to the approval of any new drug, or therapy; stem cells are certainly no exception. Despite the enormous number of research articles published each day regarding the potential of stem cells and stem cell therapy, the absence of clear, verifiable information can lead to tragedy. For example, various incidences were reported in macular degeneration patients who developed blindness, retinal detachment and intraocular bleeding, following adult stem cell-based therapy [260, 268]. Moreover, we do not yet have clear information documenting the genetic stability of ESCs, nor do we have a handle on their capacity for sustained reproducible differentiation. The use of iPSCs may overcome some of these limitations; yet, we have a long journey of research is still required to prove its safety and efficacy range. Indeed, in 2008, Yamanaka advised against the "hype" associated with iPSCs and declared that it would be quite dangerous to predict the safety of this technology with respect to clinical trials and applications [269].

Numerous factors should be considered when designing stem cell therapies. For example, an important obstacle when considering the use of umbilical cord derived stem cells is the cost of cord blood banking; these must meet the international standard regulations for the collecting, storage, and use of UC blood for transplantation [270] as well as any and all associated legal regulations [271]. At this time, the UC blood banking industry has begun to decline due to the high costs associated with its implementation. This will certainly have an impact on the future availability and therefore the use of UC derived stem cells [272].

In conclusion, the hope place in stem cells remain strong; this is certainly warranted given the opportunity to use their powerful potential to develop new cures for acute and chronic diseases. With more clinical data and improved standardization, stem cells may be safely used for treating an ever-expanding list of diseases. However, the public needs to be aware that this will take some time and that they need to be wary regarding the advertised "hype" associated with this exciting cutting-edge field. Patients are encouraged to be cautious and to look for validated and credible information before deciding to undergo an unapproved and unproven stem cell-based therapy.

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Take Home Message

- The biology of stem cells in tissue homeostasis and development has made it the prospect for the field of regenerative medicine.
- Stem cell potency is more pronounced in embryonic tissues compared to adult cells. In the adult tissues, stem cells are widely distributed throughout the body including, but not limited to, the bone marrow, adipose tissue, intestine, skin, synovial membrane, and dental pulp.
- Reprogramming somatic cells by induced pluripotent stem cell (iPSC) technology, gene editing, and applying modern techniques of nanotechnology and bioprinting have all made it possible for extensive applications of adult stem cells in regenerative medicine.
- Hematopoietic stem cells transplantation (HSCT) is already a routine practice, and has secured FDA approval for its cellular products to treat hematological diseases.
- Research is still in progress for wound healing and osteoarthritis treatment using stem cells.
- Preclinical and clinical studies showed new hope in treating incurable chronic diseases like multiple sclerosis, macular degeneration, Parkinson's Disease, and diabetes mellitus with stem cells.
- FDA, CDC, ISSCR and other stem cell societies and institutes are regularly warning about the misused stem cell therapy away from their approved applications to minimize patients' risks.
- Various types of stem cells need more clinical investigations to test their safety and efficacy before being clinically translated.
- Patients have to be cautious about the credibility of any cell-based medical application; and especially before undergoing stem cell therapy.

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