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LETTERS

Deciphering the frontiers of stem cell research in regenerative medicine

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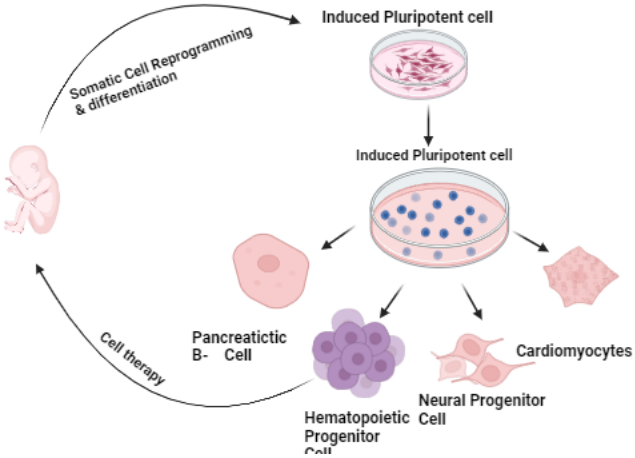
Review Article

ABSTRACT



Stem cells are the basis of the origin of differentiated cells, which again build up the fundamentals of tissue and organs. Stem cells themselves are undifferentiated and possess distinguishable characteristics such as Self-renewal, Clonality (the ability to rise from a single cell), and potency (can differentiate into many cell types). However, there are different types of stem cells with varying potencies, like pluripotent cells capable of differentiating into tissue from all 3 germ layers (endoderm, mesoderm, ectoderm). On the other hand, there are multipotent stem cells that can be differentiated into tissue derived from a single germ layer, for example: Mesenchymal stem cells, which again can be derived from various sources like bone marrow, adipose tissues, molar cells, umbilical cord, cartilage. This review also deals with how advancement in stem cell research has laid the foundation for cell-based therapies for specific diseases that remain conventionally untreated along with what challenges stand on the path of this advancement and what are the prospects. The utilization of regenerative medicines mainly deals with restoring the normal functionality of diseased tissues or organs. The applications of stem cells in regenerative medicine and therapeutics and their future scopes and challenges have also been discussed.

Keywords: Stem cells, Mesenchymal stem cells, Regenerative medicine, cell-based therapy

INTRODUCTION

Stem cells are undifferentiated cells that have the potential to differentiate and develop into over 200 distinct specialized cell types. Stem cells were first witnessed back around the 1950s, when Friedenstein et. al. isolated, cultured, and differentiated bone marrow-derived cells obtained from Guineapigs into an Osteogenic cell line.[1] In the early 1960s, Ernest A. McCulloch and James E. Till conducted pioneering experiments that revealed the existence of stem cells in the bone marrow of mice, This led to the emergence of a novel research area, and scientists have been investigating the potential of stem cells in diverse fields of medicine ever since.[2] In a recent study by a group of researchers, mice have been one of the most frequently used animals to study stem cells, mostly due to their cost-effectiveness and the ease of manipulating them genetically. However, for regenerative medicine, this animal model has subsequently failed to reciprocate human disease phenotypes, leading scientists to switch to bigger animals to design sustainable animal models, such as using pigs in replacement.[3]

Stem cells have emerged as the main source of regenerative medicine implemented for the preparation of diseased tissue and organs as mentioned in figure 1. Based on their ability to differentiate, stem cells can be classified as Unipotent, Multipotent, Pluripotent, and Totipotent. Through transdifferentiation, the zygote, the only totipotent cell in the human body, possesses the capacity to differentiate into an entire organism. Cells present in the inner cell mass of an embryo hold the ability to differentiate into cells derived from the three germ layers and hence, they are pluripotent. The pluripotent factors, OCT4, cMYC, KLF44, SOX2, and others, determine the ability of transdifferentiation as well as stemness of stem cells (from embryonic to adult cells).[3]

Based on its applications in regenerative medicine, stem cells can again be classified into Embryonic stem cells (ESCs), Mesenchymal stem cells (MSCs), Umbilical cord-derived stem cells (UCSCs), Bone marrow-derived stem cells (BMSCs) and induced Pluripotent stem cells (iPSC). However, to achieve successful transplantation status of particular stem cells, they should be able to survive, differentiate, proliferate, and be able to integrate into the circulatory system.[4] In recent developments of

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regenerative medicines, tissue engineering technologies are used in compliance with stem cell technologies to develop organoids, which are further implemented during restoring functionalities of damaged or diseased tissues and organs. Through approaches in tissue engineering, 3D scaffolds are modeled, through which properties like cell adherence and a mimic system of the targeted tissue are supported. Thus, this review is used to discuss the various applications, challenges, and future directions applicable to regenerative medicine.[5]

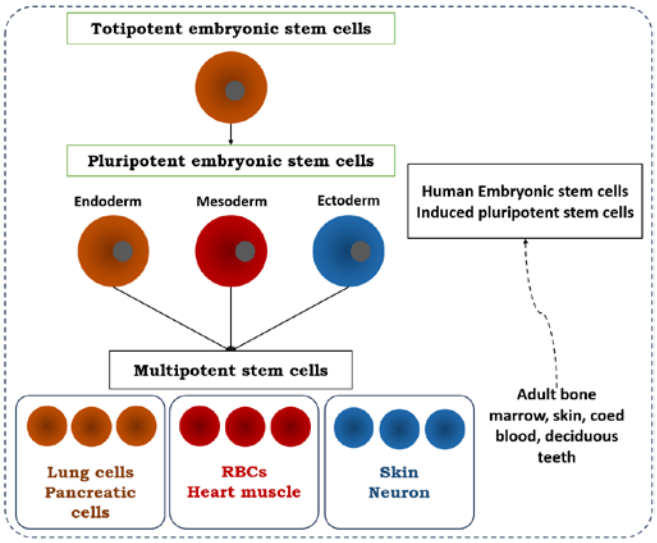

Figure 1. Hierarchy of stem cells: Totipotent stem cells have the potential of differentiating into different types of cells derived from variant sources, Endoderm, Mesoderm, and ectoderm with their potential of self-renewal. Multipotent stem cells further can be differentiated into various cell types like lung cells, pancreatic cells, RBC, Heart muscle cells, etc.

CLASSIFYING STEM CELLS

Based on their ability to differentiate:

Stem cells encompass a diverse range of cells characterized by their ability to undergo self-renewal and differentiation into specialized cell types within the body. Their classification is based on their potency, with several distinct types identified. Totipotent stem cells exhibit the highest potency and can differentiate into any cell type within the body, as well as the extraembryonic tissues that support embryonic development. These cells are exclusively present during the early stages of embryonic development before the blastocyst stage. Pluripotent stem cells, derived from the inner cell mass of the blastocyst, have the remarkable ability to differentiate into all cell types in the body. They possess self-renewal capabilities and may form teratomas, which are tumors composed of tissues originating from all three germ layers. Induced pluripotent stem cells (iPSCs) offer an alternative source of pluripotent stem cells, generated by reprogramming adult cells to acquire pluripotency. Multipotent stem cells represent tissue-specific stem cells capable of differentiating into a limited range of cell types within a specific tissue or organ. This category includes hematopoietic stem cells, responsible for generating all blood cell types, and mesenchymal stem cells, which can differentiate into bone, cartilage, and fat cells, among others. Unipotent stem cells, such as spermatogonial stem cells found in the testes, can exclusively differentiate into a single cell type. Finally, oligopotent stem cells can differentiate into a few different cell types. Notable examples include lymphoid stem cells, which can give rise to B and T cells, and myeloid stem cells, capable of differentiating into red blood cells, platelets, and various types of white blood cells.[6]

Based on sources of origin:

On this basis, stem cells are categorized into two wide categories- *Embryonic stem cells* and *adult stem cells.*

1. *Embryonic stem cells:* Embryonic stem cells (ESCs) are a type of pluripotent cells derived from the blastocyst stage of the embryo. Isolating ESC cell lines involves precise separation of the inner cell mass from the outer trophoblast, under carefully controlled conditions that support cell viability. The identification and characterization of ESCs rely on the expression of specific transcription factors such as Nanog and Oct4, which serve as markers of embryonic stem cells and are crucial for maintaining their pluripotent state. ESCs possess the ability to self-renew and are commonly cryopreserved for future use. Extensive research has focused on both mouse embryonic stem cells (mES) and human embryonic stem cells (hES). While they share fundamental stem cell characteristics, their maintenance in an undifferentiated state requires different environments. mES cells are cultivated on a gelatin layer in the presence of leukemia inhibitory factor (LIF) in serum media. Additionally, the use of a drug cocktail called 2i, which includes inhibitors to GSK3B and the MAPK/ERK pathway, has been shown to aid in preserving pluripotency in stem cell culture. Notably, studies have demonstrated that when human or mouse ESCs are transplanted into immunocompromised animal models, they can give rise to teratomas or tumors containing tissues representing all three embryonic germ layers.[7]

2. *Adult stem cells*: Adult stem cells are a crucial component of differentiated tissues and organs, exhibiting the remarkable ability to differentiate into specialized cell types. Nevertheless, in comparison to embryonic stem cells, the differentiating potential of adult stem cells is more limited. Their primary role entails residing within these specialized cellular types, ready to participate in the maintenance and repair processes when the need arises. These cells serve as vital contributors to the regenerative capacity of tissues, playing a critical role in preserving tissue integrity and functionality in times of exigency. Adult stem cells can further be classified into:

Hematopoietic stem cells (HSCs): Mesodermal-derived cells that reside in adult bone marrow are responsible for the production of mature blood cells and exhibit the remarkable ability of unlimited self-renewal. These cells predominantly exist in a quiescent state, giving rise to short-term hematopoietic stem cells (HSCs), which subsequently differentiate into either Common Myeloid Progenitors (CMP) or Common Lymphoid Progenitors (CLP). The myeloid progenitors undergo further differentiation, leading to the formation of erythrocytes, neutrophils, eosinophils, basophils, megakaryocytes, and dendritic cells. On the other hand, the lymphoid lineage

differentiates into T-lymphocytes, B-lymphocytes, and Natural Killer cells. HSCs are characterized by the absence of lineage markers but possess distinct antigenic markers on their surface. Extensive research on murine models has enabled the differentiation of murine long-term and short-term HSCs based on the presence of CD34, for instance. This comprehensive understanding of HSCs and their differentiation pathways provides crucial insights into hematopoiesis and opens up new avenues for therapeutic interventions in various blood-related disorders.[8]

Mesenchymal Stem Cells (MSCs):

Mesenchymal Stem Cells (MSCs) are a type of stromal cell with the ability to differentiate into multiple cell lineages. MSCs can be derived from various tissues such as bone marrow, adipose tissue, dental pulp, molar cells, umbilical cord, and Wharton's jelly. These cells are characterized by their plastic adherence and lack of hematopoietic antigen expression, as well as the presence of specific markers such as CD73, CD90, CD105, CD44, and CD166. MSCs from bone marrow have been shown to differentiate into adipocytes, chondrocytes, and osteocytes. MSCs are known to be immunocompetent, and their multipotency makes them potentially useful in a variety of therapeutic applications such as tissue engineering and regenerative medicine.[9]

STEM CELLS AS REGENERATIVE MEDICINE

iPSC (induced pluripotent stem cells):

iPSC was first isolated in 2006 by Takahashi and Yamanaka who experimented on skin fibroblasts and introduced factors: Sox2, Oct ¾, Klf4, and c-Myc to produce ESClike cells. Despite several ethical barriers, with the new emerging technology, iPSCs have been widely used to regenerate diseased or damaged organs and cells. Taking up the example of an ongoing preclinical trial that aims at lowering the progression rate of the disease, age-related macular degeneration (ARMD) and is successful in achieving it by producing 5-6 layers of photoreceptor nuclei by the transplantation of neuronal progenitor cells which were derived from iPSCs. In certain pathophysiological conditions, the placenta may tend to degrade, which is reversed by the reprogramming of the nucleus to form transgenic independent trophoblast-like stem cells as mentioned in figure 2. (iTSCs). Here, reprogramming of OCT4 knockout and wild-type (WT) mice fibroblast is performed using transient expression of GATA3, EOMES, TFP2C, and +/- c-myc, which finally gives rise to iPSCs. These resemble blastocysts derived from TSCs for DNA methylation. CRISPER/Cas 9 system of reprogramming iPSCs has decreased the time taken to be reprogrammed to a minimal period of about 2 weeks and this also reduces the chances of any kinds of cross-contamination as well as any genetic aberration. One of the most prevalent degenerative disorders is when serotonin neurons tend to degenerate due to various external reasons, these further may lead to bipolarity, schizophrenia, and depression-like psychiatric conditions. In this case, manipulation, and reprogramming of Wnt signaling in human iPSCs against specified culture conditions gives rise to serotonin-like neurons through specific differentiation. The development of these neurons seems to be a possible cure for Schizophrenia as they possess similar physiological functions as that of the serotonin neurons. Recent research has also successfully enabled reprogramming of skin cells into definitive endodermal progenitor cells which further differentiate and yield pancreatic Beta cells, becoming a major source of cure for diabetes. iPSCs are considered a way safer approach for cellular regeneration and the treatment of degenerative disorders.[10]

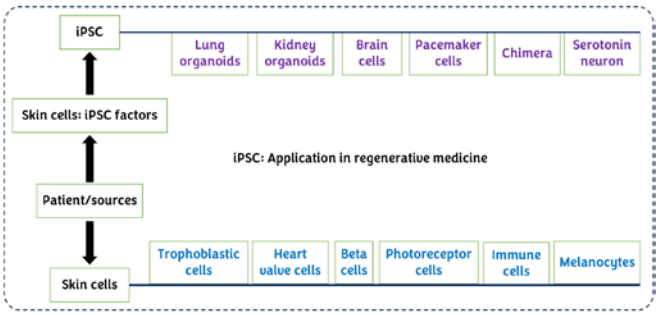

Figure 2. iPSC in regenerative medicine: The main source of it is a healthy patient from which skin cells are derived. One way is where these skin cells form iPSC, which further helps in the formation of organoids, utilized in regenerative therapy. Other than that, the skin cells may be differentiated into cells as per the need of the disease being treated. E.g., Beta cells (for regeneration of the pancreas), photoreceptors (restoring vision), or immune cells (treating blood and immune disorders).

MSC (mesenchymal stem cells):

As even discussed earlier in this review about the prospects of utilizing Mesenchymal stem cells, which are of stromal origin and are multipotent, these cells can further be subclassified based on their sources of derivation. Their uses in the form of regenerative medicines are briefed henceforth:

Bone marrow-derived mesenchymal stem cells (BM-MSCs): BM-MSCs are non-hematopoietic stem cells with vast applications in regenerative medicine and therapeutic purposes mainly due to their ability of self-renewal as well as unlimited proliferation as mentioned in figure 3. BM-MSCs are extensively studied to be further applied for the treatment of diabetes mellitus. Successful animal trials have been conducted and for the preclinical human trials, BM-MSCs were directly added to human islet cultures, which resulted in the observation that there was an increased viability in the islet cells with a noticeable production in insulin levels. There are yet several more mechanisms to be detected on the working of the BM-MSCs, but most speculations point towards the fact that they work based on the paracrine interactions.[11] After these studies were performed, a routine Glucose stimulated insulin secretion test (GSIS) at a gap of 6 months with the help of ELISA, and the viability of the islets was assayed through fluorescence microscopy. These effects of BM-MSCs on the paracrine islets can further be utilized for the development of new therapeutic procedures. However, due to the limited viability of islets cells, bioengineering approaches are being applied for the improvement of the Human islet functions.[12] BM-MSCs have other applications in regenerative medicine as well, the rarest of which can be witnessed in Periodontal diseases,

where through the application of BM-MSCs, regeneration of craniofacial bones has been tried to restore, which in traditional therapies is overlooked and solely focused on structural and functional reconstruction of bone.[13]

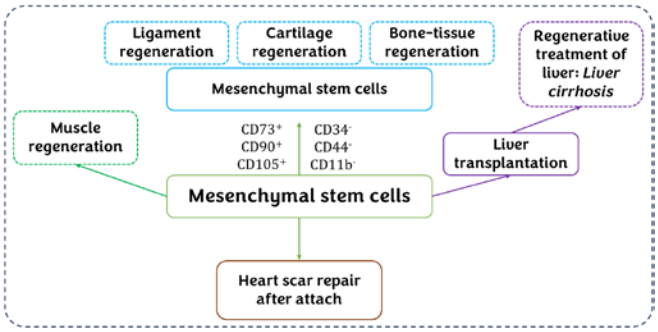

Figure 3. Mesenchymal stem cells in regenerative medicine: Multipotent in nature, these stem cells are positive for cell surface markers- CD73, CD90, CD105, CD44, and negative for markers like CD34, CD45, CD11b, CD14, CD19. These are utilized for transplantation in various animals for acting as regenerative medicine for instance, for hair reconstitution, transplantation of the liver to treat acute liver failure or liver cirrhosis, and muscle regeneration to treat muscle degenerative diseases.

Thus, organoid designs have been supplemented as a great source to deal with several degenerative diseases.

Umbilical cord-derived mesenchymal stem cells (UC-MSCs): Stem cells derived from the Umbilical cord possess lesser ethical constraints when compared to Embryonic stem cells (ESCs). The stem cells that have been derived from Wharton's jelly possess a homogenous nature which results in better differentiation into Beta cells. In an experiment, conducted on mice with the help of inserting these programmed cells has resulted in the leveling down of glucose in these mice.[14] Also, it has been observed that $CD34^+$ expression leads to outcomes with lesser chances of host versus graft rejection possibilities. UC-MSCs possess a genetic profile more similar to ESCs along with higher self-renewability when compared to BM-MSCs. The only disadvantage in the utilization of UC-MSCs is that they have to undergo prior chromosomal tests to verify there is no chance of chromosomal or genetic aberration in the donor baby. Studies prove that post-transplantation of allogeneic UC-MSCs in laryngeal patients being treated with radiotherapy restores myelination. Also, the latest case study even suggests that UC-MSCs possess the ability to treat ligament injuries, experiments conducted on pig knees by transplantation of US-MSC along with hydrogel composites led to the formation of regeneration of hyaline cartilage.[15]

ESC (embryonic stem cells):

On January 22nd, 1998, the first human ESC was isolated at the University of Wisconsin by Thomson, which further resulted in the H1 cell line. These are pluripotent, meaning that, they can give rise to more than 200 cell types and the rate of pluripotency is dependent on the functionality of the transcription factors **SOX 2, OCT4, NANOG,** and others, which are hence termed *Pluripotency factors* as mentioned in the figure 4.

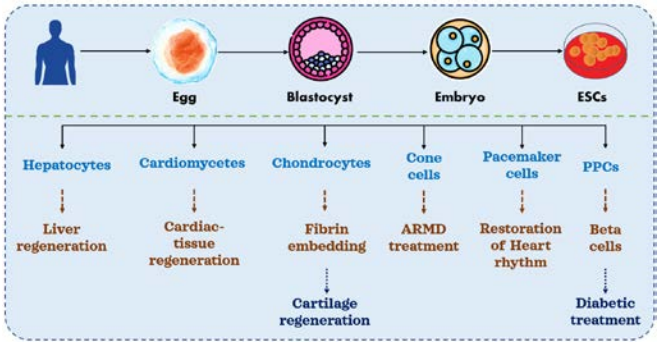

Figure 4. Embryonic stem cells in regenerative medicine: The stages of development of embryonic stem cells involve the formation of an egg, which g further forms a blastocyst, differentiating into an embryo and finally giving rise to embryonic stem cells. ESCs, sourced from ICM of gastrula, have tremendous promise in regenerative medicine. These cells can differentiate into more than 200 types of cells representing three germ layers. These then undergo specific differentiation under specific conditions to form chondrocytes, hepatocytes, cardiomyocytes, cone cells, pacemaker cells, etc. This is how we further differentiate these cells to treat a specific degenerative disorder.

Taking the case of Spinal cord injuries (SCIs) (which might be a result of multiple variants of injuries), ESCs can differentiate into a range of cells including retinal ganglion, hepatocytes, cardiomyocytes, chondrocytes, pacemaker cells, egg sperm cells, pancreatic progenitor cells, and so on. Cases of transplanting human Embryonic stem cells into paraplegic patients with spinal injury help in the attainment of improved body balance, control, and limbic movements.[16]

Another prominent case could be taken of Age-related macular degeneration (ARMD), where there is gradual degeneration of Retinal pigment with an increase in age (This is because the number of cone cells in an individual remains fixed). The COCO gene, which is expressed during Embryogenesis if incorporated in a developing embryo leads to specific differentiation of ESCs into cone cells and this involves suppression of TGF-ß, BMP, and Wnt pathways. The mechanism involves the migration of cone cells to the host retina, forming a sheet-like structure that signals recovery from ARMD. At present, Retinal ganglion cells from CRISPR Cas9-m-Cherry reporter, where during the differentiation of ESC, CRISPR/Cas9 redirected the knock-in of m-Cherry reporter into the BRN3B, gene, which is specific to RGC [17]. This step could, in the future, be used as a purification step during the generation of RGCs from other varieties of cells [18]. One of the major health problems includes the condition of Osteoarthritis, which is symptomized by stiffness of joints, caused by to wearing-away of cartilage at the joint areas. The drugs that are used to treat this work by acting on relieving the patient from the symptoms rather than initiation the reverse generation of cartilage which is worn away. Hence, no permanent solution, also, for young individuals, opting for joint replacement therapies is not feasible [18], This brings in the necessity to transplant Chondrocytes, which are derived from human Epithelial stem cells (hESC), are usually embedded in fibrin gel, and are utilized in healing cartilage defects. Defined

chondrocytes are active at the sites of transplantation which shows positive towards collagen II and SOX9.[19]

GROWTH REQUIREMENTS

Decellularized matrices provide a natural and biomimetic environment for the growth and differentiation of various types of stem cells. The growth and development of stem cells within decellularized matrices are influenced by several factors, including growth factors, gene expression, minerals, nutrients, and hormones. For mesenchymal stem cells (MSCs), the growth and differentiation can be regulated by growth factors such as transforming growth factor-beta (TGF-β), bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), and insulin-like growth factors (IGFs). These factors play crucial roles in directing MSCs towards specific lineages, including osteogenic, chondrogenic, and adipogenic differentiation. Additionally, minerals like calcium and phosphate are essential for the formation of bone tissue, which can be facilitated within decellularized matrices.[20] For neural stem cells (NSCs), the growth and differentiation can be influenced by various growth factors and gene expression. Important growth factors for NSCs include epidermal growth factor (EGF), fibroblast growth factor-2 (FGF-2), and brain-derived neurotrophic factor (BDNF). These factors promote the proliferation and differentiation of NSCs into neurons, astrocytes, and oligodendrocytes. Gene expression factors like Pax6, Sox2, and Nestin are crucial for maintaining the stemness of NSCs and regulating their differentiation potential within the decellularized matrix. Embryonic stem cells (ESCs) require specific growth factors and signaling pathways to maintain their pluripotent state and direct their differentiation. Factors such as leukemia inhibitory factor (LIF) and basic fibroblast growth factor (bFGF) play critical roles in sustaining ESC self-renewal and preventing differentiation. Additionally, the activation of specific genes like Oct4, Nanog, and Sox2 is necessary to maintain ESC pluripotency within the decellularized matrix. For hematopoietic stem cells (HSCs), growth factors and signaling molecules are essential for their self-renewal and differentiation into various blood cell lineages. Factors like stem cell factor (SCF), interleukins (IL-3, IL-6, IL-11), and thrombopoietin (TPO) support HSC expansion and promote their differentiation into erythrocytes, leukocytes, and platelets [21]. Hormones such as erythropoietin (EPO) and granulocyte colony-stimulating factor (G-CSF) can also be important for HSC growth and differentiation within the decellularized matrix. Overall, the growth and differentiation of stem cells within decellularized matrices rely on a complex interplay of growth factors, gene expression, minerals, nutrients, and hormones. Understanding and optimizing these factors are crucial for creating an optimal microenvironment that supports stem cell proliferation and directs their differentiation into desired cell lineages.[22]

Cell matrix materials

Materials used in tissue engineering and regenerative medicine can be broadly categorized into natural and artificial materials. Natural materials, such as gelatin, laminin, collagen, alginate, and hyaluronic acid, possess biological properties that are critical for maintaining the natural tissue phenotype and function. They have bioactive motifs and cell-binding domains that facilitate communication between cells and the extracellular matrix (ECM). However, natural materials often have poor mechanical properties, poorly defined compositions, and limited biochemical modification capabilities. There is also a risk of immune rejection and disease transfer when using natural materials in clinical applications.[22] Type I collagen, derived from the ECM, is a popular natural material used for 3D culture systems. It provides proper cell adhesion and migration and exhibits excellent biocompatibility, mechanical strength, degradability, and limited immunogenicity. Matrigel, derived from the Engelbreth-Holm-Swarm tumor, is another natural material that mimics the ECM by containing collagen, laminin, entactin, and important growth factors.[23] It supports the creation of self-organized neural structures resembling early-developing cerebral organoids. On the other hand, synthetic organic materials offer controlled physical and chemical properties. Polymers like polycaprolactone (PCL), poly (ethylene glycol) (PEG), and poly (vinyl alcohol) (PVA) are commonly used. These materials are inert but biodegradable, allowing cells to synthesize their own ECM within the scaffold. Synthetic polymers can be functionalized with biological components to enhance cell-matrix interactions. However, they often exhibit low hydrophilicity and can induce inflammation. Inorganic porous materials, such as graphene foam and glass microtubes, have also been utilized as scaffolds for 3D cell culture. These materials offer high mechanical stability, porosity for nutrient transport, and the ability for cells to migrate within the structure. However, their degradation and light transmission properties can be limiting factors. Both natural and artificial materials have their advantages and limitations. Research objectives and advancements could develop scaffolds that better mimic the in vivo stem cell microenvironment for successful clinical implantation.[24]

THE STAGE OF BREAKING THROUGH WITH STEM CELL THERAPY

The turning point in cell-based therapies was not quite a long time ago. Shinya Yamanaka and Kazutoshhi Takahashi for the first time in 2006, found out the possibility of being able to reprogram pluripotent cells, sourced from multipotent adult stem cells. The formation of iPSCs came into being when an experiment was performed that involved retrovirus-mediated transduction of murine fibroblasts, which consisted of OCT 3/4, SOX 2, KLF4, and c-myc (transcription factors mainly expressed in MSCs), this led to their induction into pluripotent cells or iPSCs. Similar experiments were conducted in humans in 2007 and after the success of which, this method became prevalent for research and therapy. However, recent research on lowering the risks of carcinogenicity is being performed, along with enhancing the entire conduction system to make it more biocompatible. A breakthrough in somatic cell development was achieved when John Gurdon cloned frogs by transferring nuclei derived from a frog's somatic cells into an oocyte. This experimentation proved the pluripotent nature of a somatic cell.[25]

Davis R.L. converted fibroblasts into myoblasts by expressing a gene, myogenic differentiation 1 (Myod1), originally found in myoblasts. This experimentation successfully proved the possibility of transformation of one cell lineage to another. The first allogeneic cell therapy was launched in India under the approved guidelines provided by the **Central Drugs Standards Control Organization**, in late 2022. **Stempeutics**, in collaboration with ***Alkem*** recently led to the formulation of "StemOne", which was a cell-based therapy aiming at treating Osteoarthritis, by incorporating a mechanism of repair initiation through differentiating into chondrocytes or progenitor cells of chondrocytes. Their main vision was to target people suffering from Knee Osteoarthritis, which until now had temporary treatments in use.[26]

APPLICATIONS OF STEM CELLS IN REGENERATIVE MEDICINE

ESC(s)

Various conditions such as spinal cord injuries, ARMD (age-related macular degeneration) and glaucoma, cardiovascular disease, liver injuries, diabetes, and osteoarthritis have shown potential for treatment using different types of stem cells. For spinal cord injuries, transplantation of ESCs (embryonic stem cells) to the injury site along with neurogenic factor support has resulted in the regeneration of spinal tissue and improved balance and sensation. In the case of ARMD and glaucoma, ESCs-derived cones, and retinal ganglion cells (RGCs) transplantation to the eye has led to the recovery from these degenerative conditions and restoration of vision. Cardiovascular disease has been targeted using ESCs-derived cardiomyocytes (CMs) that secrete vasculogenic factors and the use of biomaterials to coax ESCs into differentiation.[27] This approach has shown promising results in suppressing heart arrhythmias, as the CMs integrate electrophysiologically with the heart and act as pacemakers. In liver injuries, transplantation of ESCs-derived hepatocytes has demonstrated the regeneration of liver tissue, marked by the expression of specific genes such as Cytp450, PXR, CYPA4&29, HNF4-, and UGTA1. This model can also be utilized for drug screening purposes. Diabetes treatment has shown potential using ESCs-derived pancreatic progenitor cells (PPCs), which differentiate into β-cells and secrete insulin. These cells express specific genes including PDX1, GCK, and GLUT2. Transplantation of these PPCs has resulted in improved glucose levels and obesity management, offering potential treatment options for both type 1 and type 2 diabetes. Lastly, in the case of osteoarthritis, chondrocytes derived from ESCs have been transplanted to regenerate cartilage tissue. These chondrocytes, characterized by the expression of SOX9 and collagen-II genes, form cell aggregates and remain active at the transplantation site for up to 12 weeks. This approach holds promise for the treatment of cartilage injuries faced by athletes.[28]

MSC(s)

Various medical conditions and their corresponding treatments involving specific cell types and genes are outlined below as mentioned in Figure 5. Bladder deformities and infections, such as cystitis and cancer, can be addressed through the transplantation of Bladder-Derived Mesenchymal Stem Cells (BD-MSCs) with Small Intestinal Submucosa (SIS). These BD-MSCs, characterized by the expression of CD105, CD73, CD34-, and CD45-, promote bladder regeneration. For dental problems like infection, cancer, age-related issues, and accidents, the transplantation of Epithelial Stem Cells (EMSCs) and Dental Stem Cells (DSCs) within a biopolymer tissue has shown promise.[29] These transplants contribute to the regeneration of oral tissue and the development of mature teeth units. Additionally, the incorporation of vasculogenic factors aids in this process. In cases of injuries and bone degeneration, Mesenchymal Stem Cells (MSCs) can be coaxed into osteoblasts through treatments like cytochalasin-D-induced actin modeling. Furthermore, MSC infusion and transplantations help facilitate bone regeneration, reducing injury pain and potentially offering treatment for tumors. Muscle degeneration resulting from genetic factors and work stress can be addressed through the transplantation of MSCs and the controlled release of growth factors (GFs). Coaxed MSCs can be transplanted, and the use of an alginate gel protects them from immune attacks while facilitating the controlled release of GFs. This approach aids in the regeneration of heart scar tissue and muscle in a controlled manner. Alopecia, which may result from factors like age, disease, and medication use, can be treated through the transplantation of Dermal Papilla Cells (DPCs) coated with Glycosaminoglycans (GAGs). The microenvironment created by the GAG-coated DPCs promotes hair follicle regeneration, offering a potential treatment for alopecia.[30]

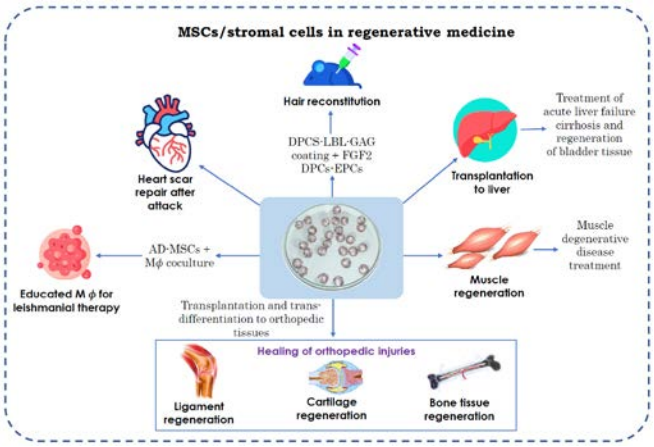

Figure 5. MSCs in regenerative medicine: mesenchymal stem cells are CD73+, CD90+, CD105+, CD34−, CD45−, CD11b−, CD14−, CD19−, andCD79a− cells, also known as stromal cells. These bodily MSCs represented here do not account for MSCs of bone marrow and umbilical cord. Upon transplantation and trans-differentiation these bodily MSCs regenerate into cartilage, bones, and muscle tissue. Heart scar formed after heart attack and liver cirrhosis can be treated from MSCs. ECM coating provides the niche environment for MSCs to regenerate into hair follicles, stimulating hair growth.

BMSC(s)

Various diseases and conditions require specific treatments or transplantation approaches. For anemia and blood cancer, injury and genetics are major causes. One treatment approach involves

a two-step infusion of Haplo identical bone marrow-derived mesenchymal stem cells (BMSCs) for reconstructing lymphoid and myeloid hematological malignancies. In the case of AIDS, HIV1 infection is targeted, and transplantation of HIV1-resistant CD4+ cells is performed. These cells express anti-HIV1 RNA, which helps restrict HIV infection and serves as an alternative to antiretroviral therapy. Blood clotting disorders, characterized by a lack of platelets, can be treated through transplantation of megakaryocyte organoids. These organoids are generated using growth factors in silk sponge and microtubule 3D scaffolds, mimicking the bone marrow environment.[31] This therapy has potential applications in burns and blood clotting diseases. Neurodegenerative diseases resulting from accidents, age, or stroke can be addressed by focal transplantation of bone marrow-derived mesenchymal stem cells (BMSCs) with the LA gene. This transplantation induces neovascularization and directs microglia colonization, leading to the treatment of neuronal damage disorders and cognitive restoration.[31] Orodental deformities caused by trauma, disease, or birth defects can be targeted using CD14+ and CD90+ tissue-resident stem and progenitor cells (TRCs). These cells accelerate alveolar jawbone regeneration and gum regeneration, aiding in the regeneration of defects in oral bone, skin, and gums. For diaphragm abnormalities resulting from accidents or birth defects, the implantation of decellularized diaphragm, derived from donor sources, serves as a replacement therapy. This therapy aims to provide a niche perfused hemidiaphragm like the natural diaphragm, allowing for improved respiratory function.[32]

iPSC(s)

Induced pluripotent stem cells (iPSCs) have shown promise in various medical applications. In the context of eye defects, including age-related macular degeneration (ARMD) and birth defects, iPSCs-derived neural progenitor cells (NPCs) can be transplanted. These NPCs form 5-6 layers of photoreceptor nuclei, leading to the restoration of visual acuity as mentioned in figure 6. Neurodegenerative disorders resulting from accidents, age, trauma, and stroke can be targeted through the transplantation of inhibitory GABAergic interneurons (iGABA-INs) and cortical spheroids. The iGABA-INs secrete GABA and the presence of the FOX1G gene can help treat conditions such as autism spectrum disorder (ASD), Alzheimer's disease, seizures, and obstinate epilepsies. In cases of liver and lung diseases caused by Alpha-1 antitrypsin deficiency (A1AD), iPSCs carrying corrected mutations of the SERPINA1 gene can be transplanted. A1AD primarily affects the liver, and the mutation leads to sensitivity to drugs. This approach offers potential treatments for chronic obstructive pulmonary disease (COPD) that affects both the lungs and liver. For diabetes treatment, iPSCs-derived pancreatic beta-cells (β-cells) can be transplanted.[33] Reprogramming skin cells into β-cells through directed epigenetic modulation and direct programming using gene regulators allows for insulin production. This approach can be used for the treatment of type 1 and type 2 diabetes. In cases of lung degeneration caused by conditions such as tuberculosis, cancer, and fibrosis, biomaterial-coaxed iPSCs can be transplanted. These miniature iPSCs form lung structures resembling airways and alveoli, enabling drug testing, and offering potential for the regeneration of lung tissue. Finally, in the context of immune diseases, transplantation of iPSCs corrected using Oct4 and Nanog genes can be performed.[34] The use of CRISPR-Cas9 technology allows for the generation of iPSCs in a single step, and iPSCs modified to differentiate into macrophages (iPSCs-Mϕ) can resist HIV1 infection. This approach holds potential for immunotherapy of immune diseases such as severe combined immunodeficiencies (SIDs), HIV1, and others.[35]

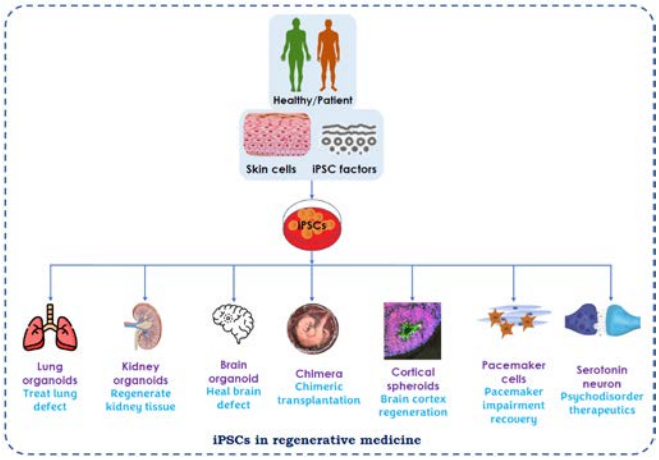

Figure 6. iPSCs in regenerative medicine: using the edge of iPSCs technology, skin fibroblasts and other adult tissues derived, terminally differentiated cells can be transformed into ESCs-like cells. It is possible that adult cells can be transformed into cells of distinct lineages bypassing the phase of pluripotency.

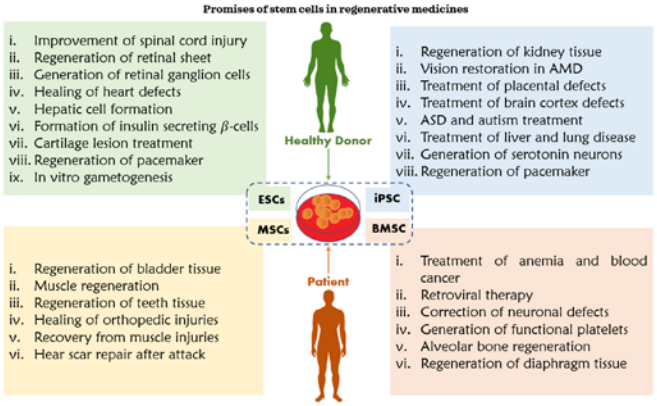

Figure 7. Promises of stem cells in regenerative medicine: the six classes of stem cells, that is, embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), bone marrow stem cells (BMSCs), and induced pluripotent stem cells (iPSCs), have many promises in regenerative medicine and disease therapeutics.

CHALLENGES TOWARDS THE USE OF STEM CELLS IN REGENERATIVE MEDICINE

Isolation of Stem Cells

Bone marrow stem cell extraction involves removing bone marrow from the hip bone or the sternum using a needle which is generally considered safe, but it can cause pain, swelling, and bruising at the extraction site. In some cases, bone marrow

extraction can also lead to infections and bleeding while the number of stem cells extracted from bone marrow is limited, and the quality and quantity of extracted cells can vary based on the age and health of the donor. Bone marrow aspiration may not yield a sufficient number of stem cells, especially in older patients, which can limit its usefulness in regenerative medicine. There is a risk of contamination during the extraction process, which can affect the quality and viability of the stem cells obtained. A study conducted examined the challenges associated with bone marrow aspiration for stem cell therapy as mentioned in figure 7. The study found that the yield of stem cells obtained from bone marrow aspiration can vary significantly based on the patient's age and health status. The researchers also noted that contamination and low yield are significant challenges that need to be addressed to improve the effectiveness of stem cell therapy using bone marrow aspiration.[36] Adipose tissue stem cell extraction involves removing adipose tissue from the abdomen or thighs using a liposuction procedure which is generally considered safe, but it can cause pain, swelling, and bruising at the extraction site. In addition, the quantity and quality of extracted cells can vary based on the age and health of the donor.[37] Embryonic stem cell extraction involves harvesting stem cells from embryos, which can raise ethical concerns while requiring a complex extraction process requiring specialized knowledge and techniques. The extraction process can also damage the embryos, reducing their viability and effectiveness. The number of stem cells obtained from umbilical cord blood is limited, which can restrict its use in regenerative medicine. The stem cells obtained from umbilical cord blood may require HLA matching to reduce the risk of rejection by the patient's immune system, which can limit their usefulness. The cost of umbilical cord blood collection and storage can be high, which can limit its availability to patients. A study conducted by Bertrand et al. (2020) examined the challenges associated with umbilical cord blood collection for stem cell therapy. The study found that the limited availability of stem cells obtained from umbilical cord blood can be a significant challenge, especially for larger patients or those with higher stem cell requirements. The researchers also noted that cost and HLA matching are significant challenges that need to be addressed to improve the accessibility and effectiveness of umbilical cord blood collection for stem cell therapy. The processing time required to extract and isolate stem cells from adipose tissue can be longer than other methods, which can limit its usefulness in emergencies. Induced pluripotent stem cell extraction involves reprogramming adult cells to become stem cells, the process of which can be complex and requires specialized techniques while the quality and quantity of extracted cells can vary based on the type and age of the original cells.[38] The isolation of adult stem cells from various tissues and organs poses several challenges. One major hurdle is the rarity of these stem cells. Hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), for example, are present in very low numbers within their respective tissues. The difficulty lies in obtaining a sufficient quantity of stem cells for therapeutic purposes. Additionally, the isolation process itself requires complex techniques.[39] Methods such as fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and density gradient centrifugation are employed, but they can be time-consuming, costly, and may not yield a high purity of stem cells. Furthermore, identifying and characterizing adult stem cells is challenging due to their heterogeneity and lack of specific surface markers. While certain markers are associated with stemness, they are not exclusive to stem cells, necessitating additional criteria for confirmation. Another significant challenge stems from the dependency of adult stem cells on their in vivo niches within tissues. Isolating them disrupts this native environment and can impact their behavior and functionality. Replicating these complex niche conditions in vitro remains a challenge. Moreover, different tissues may require specific isolation protocols, further complicating the process. Achieving high purity and minimizing contamination with other cell types or non-viable cells is crucial for safe and effective stem cell therapies.[40]

Growth and Maintenance of Stem Cells

Stem cells present several challenges in their use for regenerative medicine. Firstly, stem cells are a heterogeneous population of cells, and their quality and characteristics can vary based on the source of the cells, culture conditions, and differentiation protocols. Achieving homogeneity and purity of the cell population is a challenge to obtain consistent outcomes. Secondly, stem cells have a limited lifespan in vitro and can undergo senescence over time, reducing their therapeutic potential. Optimizing culture conditions, such as growth factors and extracellular matrix components, can improve stem cell viability and therapeutic potential. Thirdly, stem cells can undergo genetic and epigenetic changes during culture, affecting their stability, differentiation potential, and safety for clinical use [41]. Reducing the use of xenogeneic components and using defined culture media can help minimize these changes and improve stem cell therapy's safety and efficacy. Fourthly, immune rejection can occur in stem cell therapy, and strategies to prevent it, such as immunosuppressive drugs, can have adverse side effects, limiting clinical application. Engineering stem cells to express immunomodulatory factors or using immunomodulatory drugs can improve their survival and integration into host tissue. Finally, the scalability of stem cell production is a significant challenge as producing large numbers of cells is often labor-intensive, time-consuming, and expensive. Using bioreactors and automation can improve the scalability and cost-effectiveness of stem cell production, enabling their widespread use in regenerative medicine.[42] Stem cell therapy has great potential in medical practice, and preclinical studies related to stem cell therapy have been extensively conducted. However, obtaining specialized cells in sufficient amounts and high purity for a specific therapeutic application remains a significant challenge, despite the rapid evolution in differentiating stem cells into many different cell types. A thorough understanding of the mechanisms from stem cell culture to transplantation into a patient is crucial to realizing stem cell applications in the clinic. Identifying stem cells, especially adult stem cells, is challenging because there is no consensus regarding the characteristic surface epitopes that can be used to identify the cells.[43] Maintaining stemness of stem

cells in vitro is also essential as stem cells tend to differentiate into lineage cells spontaneously. The immunogenicity of cell types and tissues differentiated from pluripotent stem cells is another contentious issue in regenerative medicine. Achieving the directed differentiation of stem cells is yet another challenge, as tissue microenvironmental cues are crucial for directing stem cell fate in vivo in both physiological and pathological conditions. Researchers have tried to discover key factors that direct stem cell differentiation, and integrating soluble factors or ECM components into a biophysical cue-based induction regime can enhance the efficiency, efficacy, and sustainability of the inducing effect. However, a complete understanding of this process is yet to be reached.[44] Mouse and in vitro models of human disease have limitations when designing effective human therapies. iPSCs have enabled the modeling of human heterogeneity in cardiac and neurodegenerative disorders and the identification of distinct and common aberrant gene pathways. However, one of the most important problems for clinical trials in regenerative medicine is that "compelling evidence of therapeutic effectiveness has generally not been delivered by the field." To increase the chances of success in future clinical trials, preclinical studies should show sufficient scientific evidence. The lack of an effective method for inducing immune tolerance of allogeneic cell transplants remains a serious roadblock to cell therapies. Culture systems can add heterogeneity to the phenotype and genotype, thus complicating selection criteria for transplantation. The lack of standardization of the materials for potential use in patients raises concerns for regulators and regarding therapeutic approval.[45]

Scaffolds

Decellularization is a process to obtain an acellular extracellular matrix (aECM) scaffold by removing cellular components from living tissue using chemical or physical methods. The resulting aECM scaffold is a three-dimensional structure that can be used as a natural scaffold in tissue engineering and regenerative medicine. It retains the structural integrity and biochemical properties of the native tissue, exhibiting bio-inductive properties for cellular attachment, migration, proliferation, and function. Decellularization has been successfully applied to various mammalian organs, preserving the native vasculature for adequate perfusion. On the other hand, 3D printing offers automated fabrication, reproducibility, increased resolution, and mass production potential, finding applications in orthopedics, maxillofacial surgery, and tissue engineering. However, challenges remain in producing complex structures and maintaining viable tissues with vascular networks. Future advancements in bioprinting techniques, including the use of multipolymer constructs and multiple cell lines, hold promise for overcoming these limitations and achieving adequate tissue perfusion.[46]

The engineering of tissues and organs requires materials that can replicate both their structure and function. Decellularization and recellularization techniques have emerged as promising approaches for achieving this goal. Decellularization involves the removal of immunogenic cells from tissues and organs while preserving the native extracellular matrix (ECM). The decellularized ECM can then be used as a bioink in 3D bioprinting, providing tissue-specific properties that promote cellular proliferation and differentiation. 3D bioprinting techniques based around the design and fabrication of tissues/organs, currently rely on standard methods, which can be difficult given the significant variations among cells obtained from different individuals, leading to differences in proliferation and differentiation.[47] Technical challenges also exist, such as the speed of the bioprinting process and the biocompatibility of materials used. Furthermore, the printing of multiple biomaterials and cells in precise locations within a scaffold or graft may require a combination of different bioprinting strategies, which subsequently need to be cultured in a bioreactor for maturation, allowing cells to deposit extracellular matrix (ECM) and synthesize necessary biomolecules like growth factors. One of the major challenges that 3D bioprinting addresses in regenerative medicine and tissue engineering is vascularization. The creation of vascularized tissues has been successful in animals and human tissues, showing promising outcomes in bone formation. However, for the resulting constructs or grafts to be suitable for human use, high-quality 3D bioprinting is essential, and stringent quality controls equivalent to those for human drugs must be implemented at each stage. While most trials have been conducted in animals, gaining approval from regulatory authorities like the FDA or the European Medicines Agency is crucial for translating 3D bioprinted constructs and grafts into clinical practice. Moreover, challenges such as detergent retention and potential degradation of the ECM after transplantation need to be addressed.[48] Synthetic scaffolds, on the other hand, fail to fully replicate the properties of native tissues. Hydrogels, which are biodegradable and exhibit similar characteristics to tissues, have gained attention in tissue engineering. Combinations of natural and synthetic biomaterials have been explored to enhance cell adhesion and proliferation. The use of composite biomaterials or scaffolds holds great potential for advancing regenerative medicine. The function of seeded cells in these scaffolds is still a topic of debate, with some evidence suggesting that they primarily induce inflammation, leading to the recruitment of host cells for tissue regeneration. Noninvasive imaging technologies, such as MRI and CT, have enabled the creation of patient-specific replacement tissues based on individual body measurements. These advancements pave the way for personalized scaffold fabrication and the development of next-generation therapies in regenerative medicine.[49] Nanotechnology combined with stem cell therapy encounters various challenges, including cytotoxicity and safety concerns of nanoparticles (NPs), as well as their impact on stem cell differentiation. Understanding the communication between cells and nanomaterials, their metabolism within cells, and their effects on cell function is crucial but complex, hindering the monitoring of nanotechnology in cell-based therapies. Additionally, there are difficulties in processing, characterizing, and tailoring 3D nanostructures for tissue engineering.[50] The substitution of gene nanotechnology-based stem cell therapy with traditional gene delivery systems is being actively explored. Despite these obstacles, nanotechnology holds the potential to

drive advancements in cell-based and stem cell-based therapies, particularly for the treatment of degenerative diseases in the future. Overcoming challenges related to multifunctional nanostructures, packaging, characterization, interface problems, nanomaterial quality, and regulating their behavior on stem cell surfaces are also critical for effective stem cell nanotechnology techniques in regenerative medicine applications.[51]

Apoptosis and Factors Affecting Stem Cell Renewal

An enhanced paradigm to support tissue repair and functional recovery may be feasible with stem cell-based therapy. The high rate of transplanted stem cell death complicates both the prognosis and outcome of transplantation as mentioned in the fgure 8. It is therefore imperative to investigate the mechanisms underlying stem cell death, including but not limited to ischemia/hypoxia, reactive oxygen species, inflammatory response, excessive autophagy, and activation of the apoptotic cascade. Emphasizing the pertinent metabolic pathways may be a helpful strategy to boost stem cell viability and transplant success. Under hypoxic culture, it has been demonstrated that a hypoxia-inducible factor-1α (HIF-1α) mechanism increases the expression of CXCR4, CXCR7, and SDF-1 in stem cells [52]. The transcriptional circuits of Klf4, c-Myc, and Nanog have a role in the connection between reprogramming and the emergence of cancer.[53] This reprogramming seems to be done piecemeal, with the trio combining to form a complex, oxygen-dependent transcriptional machinery. Cell survival under hypoxia and cell cycle activation are regulated by c-Myc, which is in turn controlled antagonistically by Nanog and Klf4.[54]

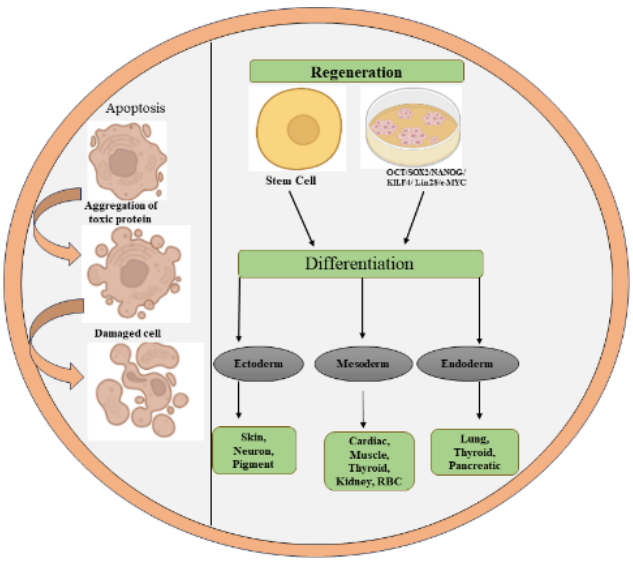

Figure 8. Apoptosis significantly decreases the accumulation of damaged protein inside the cell. Cellular differentiation is the process in which a stem cell changes from one type to a differentiated one.

The fact that a more complex network of cell death is given more attention than a single, significant pathway in deciding the destiny of stem cells highlights the challenges in identifying processes and possible targets for treatment. Cell-based treatments have long been anticipated to enhance tissue repair and speed up the healing process. Because they are a cellular reserve, stem cells (SCs) may create, maintain, repair, and even regenerate a wide range of tissues, each endowed with the special powers of self-renewal and differentiation. Stem cells (SCs) have become the most ideal source for cell-based therapies due to their ability to differentiate into various cell types and produce chemicals that are impacted by their environment. Based on their developmental stage, three types of stem cells can be identified: induced pluripotent stem cells (IPSCs), adult stem cells (ASCs), and embryonic stem cells (ESCs). The source of ESCs is the mass of a blastocyst's inner cell. Ethical constraints apply to the use of ESCs in therapy. Unlike ESCs, IPSCs generated from mature body cells can differentiate into pluripotent SCs through regulation, making them a renewable source of various cells and tissues [55]. ASCs, sometimes referred to as somatic SCs (SSCs), are found in adult tissues such as neural SCs (NSCs), hematological SCs (HSCs), mesenchymal SCs (MSCs), and cutaneous SCs. Numerous trials have demonstrated the potential of using ASCs for disease treatment. Bone marrow is typically used to treat strokes. There are two distinct situations in which cell replacement and regeneration take place: the replenishment of exhausted cells during tissue homeostasis, referred to as homeostatic growth, and the response to external damage, injury, or amputation, also known as epimorphic regeneration. Model animals with exceptional regeneration abilities include the livers of vertebrates, amphibians, planarians, and hydra. Furthermore, damaged or worn-out cells can be substituted with new ones in several animal organs, including the kidney, skin, stomach, muscle, and even the human brain system[56]. Blastema development typically plays a role in the regeneration response, despite its complexity. Through apoptosis, a morphologically unique type of programmed cell death, the majority of animal cells are capable of self-destruction. Both tissue homeostasis and development depend on the appropriate control of apoptosis, and the initiation and spread of cancer are facilitated by apoptosis inhibition. Caspases, a family of cysteine proteases found in almost all cells as minimally active zymogens, are one of the key steps in the execution of apoptosis. Proteolytic cleavage of the zymogen results in caspase activation in response to a variety of stimuli, including developmental signals and different types of chronic cellular stress or injury, such as viral infection, DNA damage, hypoxia, increased reactive oxygen species, loss of cellular adhesion, and accumulation of unfolded protein excitotoxicity, shear stress, cytoskeletal damage, and other function [57]. An indirect cause of apoptosis is necrosis, which is the body's response to severe physical injury in cells. In each of these cases, apoptosis appears to function as an efficient cellular quality control mechanism, removing undesired, unhealthy, and occasionally harmful cells from the body. Many tissues can withstand an unexpectedly high level of cell death, after which they speed up cell division and regeneration to replace the lost tissue. For example, even after more than half of their cells have been destroyed, growing Drosophila imaginal discs—the larval precursor structures of adult legs, wings, and eyes can create a normal-sized and patterned organ. In a similar vein, 75% of a full-sized mammalian liver can recover following removal.

Depending on the kind of tissue damage, these regeneration mechanisms involve several stages, such as wound healing, the formation of proliferative blastema cells, differentiation, and patterning. In 1988, scientists hypothesized that apoptosis might be a key factor in promoting cell proliferation during tissue regeneration.[58] This surprising discovery has been validated by studies conducted on various model species, including Drosophila, Hydra, planarians, Xenopus, newts, and mice.[59] In particular, apoptotic cells control the proliferation component of regeneration, including blastema cell development. The term "apoptosis-induced compensatory proliferation" refers to this occurrence. The ability of stem cells to self-renew is an essential mechanism for maintaining their populations. For example, self-renewal is not the same as quiescence entry since quiescent stem cells in the intestinal epithelium proliferate continuously without going into quiescence. Alternatively, quiescent stem cells can only self-renew by going through one cell division, which requires them to enter and exit the cell cycle. Choose between continuing to proliferate, exiting the cell cycle and differentiating, or returning to quiescence are the decisions that activated stem cells must make. Also, the effect of drug treatment during stem cell regeneration and differentiation is a great achievement in the field of stem cell therapy.[60]

Disease modelling and finding drugs are just two of the potential therapeutic uses for stem cells, along with cell transplantation and regenerative therapy.[61] Allogeneic rejection, limited cell supply, and stem cell growth regulation are a few of the obstacles that need to be overcome before this potential may be realized. To do this, it would be essential to create effective techniques for manipulating stem cell fate and to learn more about how cells work controlling stem cell potential.

Ethical Issues and challenges for stem cell-induced Regenerative Therapy

Embryonic stem cell research has always been ethically controversial because of the destruction of human embryos in the process of deriving new stem cell lines. This has hindered the development of new stem cell-based therapies. Fortunately, induced pluripotent stem cells (iPSCs) do not raise ethical concerns as they do not use embryos or oocytes. Adult stem cells are also widely used in research and clinical applications. However, these cells have limitations as they cannot expand much in vitro and have not been definitively shown to be pluripotent. Blastocysts are currently derived from excess fertilized eggs obtained from in vitro fertilization clinics, which is a limited source. Alternatively, nuclear cloning, involving the introduction of a nucleus from a donor cell into an enucleated oocyte, can be used to generate an embryo with a genetic makeup that is 99.5% identical to that of the donor. The controversy over stem cell research is based on philosophical, moral, or religious views, including the moral status of the embryo and the possible use of savior siblings as a source of embryonic stem cells. The ethical debate is mainly about benefits and safety, where a higher risk is acceptable if there is a bigger benefit. Safety concerns are for the individual and the wider community [62]. As non-ESC use becomes more widespread, acceptance of ESCs treatments may increase, and eventually, ESCs might be introduced for treating several conditions, including diabetes, spinal cord injuries, liver injuries, and heart transplantation. The potential benefits of stem cells for regenerative medicine have led to the exploration of various sources for clinically applicable stem cells. However, certain manipulations of stem cells, including embryo destruction and cell reprogramming, have raised deep ethical concerns. The application of human embryonic stem cells (hESCs) is particularly problematic, as they cannot be obtained without destroying a human embryo. To address this issue, human induced pluripotent stem cells (hiPSCs) were generated by reprogramming somatic cells to mimic hESCs, but this technique also poses ethical concerns, such as the potential for abnormal reprogramming and tumor generation during stem cell therapy. On the other hand, adult stem cells, such as bone marrow stromal cells (BMSCs) and adipose-derived stem cells (ADSCs), are gaining attention as they can be obtained without ethical issues. The development of techniques to produce stem cells suitable for research and therapeutic purposes without the destruction of human embryos may help to conclude the ethical debate surrounding stem cell use. Another stem cell technology, somatic cell nuclear transfer (SCNT), used to create nuclear transfer stem cells (NTSCs), also faces ethical and technological challenges. SCNT has been controversial due to concerns about its potential for human cloning. However, efforts have been made to establish a global governance framework to maximize benefits while avoiding ethical concerns through knowledge sharing and feasibility testing. Induced pluripotent stem cells (iPSCs) offer distinct advantages over hESCs and NTSCs. They eliminate ethical concerns, reduce the risk of immune rejection, and provide the opportunity to obtain large quantities of cells for personalized medicine. Despite challenges in clinical development, the first successful clinical case of transplanting iPSC-derived retinal cells demonstrated their feasibility and clinical potential while addressing ethical concerns. Recent controversies surrounding unauthorized human germline gene editing using CRISPR technologies have ignited ethical debates. The potential of gene editing in human embryos to prevent or correct diseases is significant, but strict regulation and societal approval are essential to ensure responsible use.[63] In the future, research in the field of stem cells and gene editing will require the involvement of governmental bodies, society, and the scientific community to establish proper regulations, ethical guidelines, and permissions to navigate these complex and evolving technologies responsibly.[64] Innovative therapeutic solutions must be developed to reduce the adverse impacts of the complex and multidimensional processes of aging and frailty. Mesenchymal stem cells (MSC) contribute to the maintenance of tissue homeostasis through a "galaxy" of tissue signals, including proliferative, anti-inflammatory, and antioxidative stress capabilities, in addition to proangiogenic, anticancer, antifibrotic, and antibacterial activities. MSCs are not age-resistant, though.[65] Three MSC-based approaches have been proposed: replace, rejuvenate, or get rid of the senescent MSC. This concept rests on the potential use of MSCs, which are composed of substances that have the same biological effects as their parent cells, such as cytokines, growth factors, and cells, and include growth factors,

cytokines, and extracellular vesicles. Thus, this tactic enables us to avoid the drawbacks of therapy. Ex vivo-expanded stem cells have long been a mainstay of biotherapeutics, but interest in them is growing due to their potential to improve tissue regeneration and treat incurable diseases. But using external cellular resources to develop restorative treatments for large numbers of patients has become a serious concern, for safety and cost reasons alike.[66] Since cell biology research has advanced over the past 20 years, there has been a greater possibility to use endogenous stem cells during wound healing processes. Regenerative research and therapy that emphasizes endogenous cell recruitment has been particularly prompted by new insights into the homing and migration of stem cells. Stem cells use chemoattractant gradients to direct their trafficking and homing, and cell features play a major role in determining which wounded or diseased tissues they target. However, only a small percentage of therapeutically supplied cells can home and engraft within the wounded tissue. Stem cells, and MSCs in particular, have been demonstrated to possess migratory and targeting capacities.[67] In the lack of natural stimuli, MSCs exhibit modest levels of chemokine receptors, which vary according on the donor's age and the tissue of origin. More reduction in these receptors occurs during in vitro expansion, which is necessary to obtain several clinically relevant cells.

FUTURE DIRECTIONS

The regeneration potential of ASCs has paved the way for its wide-ranging application in regenerative medicine, marked as a means of curative treatments for diseases not curable through conventional approaches. At present, the uses of these cells are narrowed down to a state where iPSCs are mostly utilized to develop cell-based therapies for treatment purposes, whereas extensive research on ESCs is being conducted to analyze the basis of organogenesis and cellular development. Recent studies are being performed, keeping in focus a lot of diseases of which, cardiofunction reconstruction is one of the topics to be prioritized. Coping with the increase in cardiac diseases, of which, the most prevalent being Myocardial infarction, there has been a rise in the need for cardiac reconstruction, which is again being made possible with the help of iPSCs, thus establishing its pluripotent nature. This mainly focuses on the production of standardized pluripotent cell lines for specific patients. However extensive studies are still being performed on analyzing the exact mechanism behind the pathophysiology chosen by the specialized stem cells. Despite this, it is undeniable that the future of regenerative medicine rests in the hands of stem cell-based therapies.[68] Through the performance of various preclinical trials, it has been evident that the prospects of cell-based therapies hold great potential, in terms of various diseases like Diabetes mellitus, cirrhosis, Crohn’s disease, pulmonary fibrosis, and other neurodegenerative diseases. A major issue of immune rejection was faced before the discovery of iPSCs, but with the implementation of iPSCs and MSCs in research, this issue was surpassed with ease. Also, the ethical concerns regarding the usage of ESCs can be avoided too, with the utilization of iPSCs in turn, as recent research has led to the recurrent gain in pluripotency of iPSCs even when derived from old and diseased patients or individuals.[69] While 74.8% of clinical trials involved induced pluripotent stem cells (iPSCs), compared to 25.2% with embryonic stem cells (ESCs), a different pattern emerges when focusing on interventional studies. In the majority (73.3%) of interventional trials, ESCs were utilized, while iPSCs were used in only 26.7% of cases. However, considering the increasing number of iPSC trials, a shift towards interventional iPSC studies may be imminent. The development of non-integrating transgene-free reprogramming methods may address concerns of in situ carcinogenicity due to viral particles. Geographically, there are notable differences, with observational studies predominantly conducted in the USA (41.6%) and France (16.8%), while interventional studies are primarily carried out in Asian countries such as China (36.7%), Japan (13.3%), and South Korea (10.0%). This variation can be attributed to legal and regulatory restrictions, research policies, and ethical regulations. Ophthalmic diseases were a significant focus, with 24.4% of all studies targeting sense organ diseases, particularly the eyes (63.3% of interventional trials). Notably, there were limitations in interventional studies, including a lack of placebo or untreated control groups (0% and 6.7% respectively), limited long-term follow-up, and inadequate reporting in peer-reviewed journals. While iPSC research has garnered attention, it has yet to be extensively explored in clinical investigations, and future advancements will determine its clinical applicability and ability to overcome associated challenges.[70] Ongoing research aims to improve the efficiency and safety of iPSC generation and optimize their differentiation into various cell types for therapeutic applications. Gene editing technologies like CRISPR-Cas9 have opened up new avenues for precise modification of stem cells' genetic material. This enables the correction of disease-causing mutations, enhancement of therapeutic properties, and better control over the differentiation process. Continued advancements in gene editing techniques will enhance the therapeutic potential of stem cell therapies. Stem cells with biomaterials and three-dimensional (3D) printing technologies allow the creation of complex tissues and organoids. These engineered constructs can mimic the structure and function of native tissues, providing valuable models for drug screening and personalized medicine. Further advancements in tissue engineering techniques will enable the creation of functional organs for transplantation. Stem cell behavior is influenced by their surrounding microenvironment, known as the stem cell niche. Understanding and optimizing the components of the niche, such as biochemical cues, physical properties, and cellular interactions, can enhance stem cell survival, proliferation, and differentiation. Manipulating the stem cell niche through biomaterials, growth factors, and supportive cells will contribute to improved stem cell therapies. of different stem cell types, or combining stem cells with other treatment modalities, such as gene therapy or immunotherapy, can synergistically enhance therapeutic outcomes. The use of supportive scaffolds, biomolecules, and growth factors alongside stem cells can create a conducive environment for tissue regeneration. Future advancements will focus on identifying optimal combinations

and developing strategies for effective and safe combinatorial therapies.

The lack of congruent regulatory frameworks and limitations of patents in the stem cell field have led to the exploration of alternative opportunities in intellectual property (IP) to overcome current challenges. In jurisdictions where patents are not effective for protecting stem cell innovations, mechanisms like confidentiality, trade secrets, trademarks, utility models, copyrights, or creative commons are being considered as alternatives to fill the gap. Novel business models that leverage stem cells for cutting-edge technologies, such as 3D printing, bioprinting, and genomic editing, require a comprehensive IP-protected toolset to address the needs of rapidly aging societies.[71] Translating stem cell research into economic and social benefits necessitates collaboration between researchers and the business world, with bridge-builders connecting the two. Strengthening the conceptual framework and metrics around knowledge transfer, along with exploring diverse avenues like startups, spin-offs, and SMEs, can enhance the commercialization and application of stem cell therapies. Analyzing patent activity reveals ongoing growth and innovation in the field, with concentrations of patent protection in regions like the USA, European Union, and Australia. Regulatory updates,[63] innovative financing models, and alternative forms of IP can contribute to achieving the ultimate goal of stem cell therapies, promoting wealth and well-being in society, particularly in the post-pandemic recovery phase.[62]

CONCLUSION

Stem cells have emerged as a promising tool in regenerative medicine due to their ability to differentiate into various specialized cell types. They offer immense potential for treating a wide range of diseases and injuries that were previously considered incurable. However, the field of stem cell research and its application in regenerative medicine still face several challenges. One of the major challenges is achieving consistency and purity of stem cell populations. Stem cells are heterogeneous, and their characteristics can vary depending on the source, culture conditions, and differentiation protocols. Standardization and optimization of these factors is crucial to obtain consistent and reliable outcomes in stem cell therapies.

Another challenge is the limited lifespan of stem cells in vitro and their tendency to undergo senescence. Overcoming this limitation is essential to harnessing their full therapeutic potential. Improving culture conditions, including the use of growth factors and extracellular matrix components, can enhance stem cell viability and functionality. Genetic and epigenetic changes that occur during stem cell culture pose another challenge. These changes can impact the stability, differentiation potential, and safety of stem cells for clinical use. Minimizing the use of xenogeneic components and utilizing defined culture media can help mitigate these changes and enhance the safety and efficacy of stem cell therapies. Immunological rejection is a significant hurdle in stem cell therapy. The immune system can recognize and attack transplanted stem cells, limiting their survival and integration into the host tissue. Strategies such as engineering stem cells to express immunomodulatory factors or using immunomodulatory drugs can improve their survival and enhance their integration into the host tissue, thus reducing the risk of rejection. Scalability of stem cell production is also a challenge that needs to be addressed. Current methods of producing large numbers of stem cells are labor-intensive, time-consuming, and expensive. Developing efficient and cost-effective approaches, such as bioreactors and automation, can improve scalability and facilitate the widespread use of stem cell therapies.

Despite these challenges, the prospects of stem cells in regenerative medicine are promising. Ongoing research, particularly in the field of induced pluripotent stem cells (iPSCs), offers opportunities for developing personalized cell-based therapies. iPSCs have the advantage of avoiding ethical concerns associated with embryonic stem cells, making them a valuable tool for studying diseases and developing treatments. Extensive studies are being conducted to understand the mechanisms of various specialized stem cells and their potential applications in different diseases. Furthermore, the use of stem cells in cardiac regeneration and reconstruction holds great promise. With the increasing prevalence of cardiac diseases, such as myocardial infarction, stem cell-based therapies using iPSCs offer a potential solution. The pluripotent nature of iPSCs allows the production of standardized cell lines tailored to specific patients, paving the way for personalized treatments. Ethical concerns surrounding stem cell research, particularly embryonic stem cell research, have hindered its progress. However, the development of alternative approaches, such as iPSCs and adult stem cells, has alleviated some of these ethical concerns and expanded the possibilities for stem cell-based therapies. While challenges remain, the role of stem cells in regenerative medicine is increasingly recognized as a promising avenue for treating previously incurable diseases and injuries. Addressing the existing hurdles and further advancing our understanding of stem cell biology will be instrumental in unlocking their full potential for clinical applications, ultimately revolutionizing the field of regenerative medicine.

Abbreviations:

MCS: Mesenchymal Stem Cells
iPSCs: induced pluripotent stem cells
GFs: Growth Factors
ESCs: embryonic stem cells
FACS: fluorescence-activated cell sorting
MACS: magnetic-activated cell sorting
BMSCS: bone marrow stem cells
BM-MSCs: Bone marrow-derived mesenchymal stem cells
SCNT: Somatic cell nuclear transfer
hESCs: Embryonic stem cells
ARMD: Age-related macular degeneration

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest for publication of this review work.

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