2019 SCIENCELINE
Journal of Life Science and Biomedicine
J Life Sci Biomed, 9 (2): 52-63, 2019

ISSN 2251-9939

Current status of stem cell therapy

Mastewal Birhan[1], Amebaye Kenubih[2], and Muluken Yayeh[3]

Department of veterinary Paraclinical studies, College of Veterinary Medicine and Animal Sciences, University of Gondar, Gondar, Ethiopia
Corresponding author's Email: maste675@gmail.com; ORCID: 0000-0002-0984-5582

ABSTRACT

Introduction. Stem cells have the extraordinary potential to develop into many diverse cell types in the body during early life and growth. Significant progress has been made in understanding the biochemical and metabolic mechanisms and feedback associated with different stem cells response. Some of the challenges concerning transplanted embryonic stem cells and mesenchymal stem cells are immune-mediated rejection, senescence-induced genetic instability or loss of function, and limited cell survival.
Aim. The aim of this review, is to recapitulate the recent status and information about the use of embryonic stem cells and mesenchymal stem cells for research into how cells and tissues of the body grow and develop, and potentially useful for curing disease.
Results. Stem cell therapy efforts are currently underway for virtually every type of tissue and organ within the human body. Because the current status of stem cell incorporates the fields of cell transplantation, materials science, and engineering, personnel who have mastered the techniques of cell harvest, culture, expansion, transplantation, and polymer design are essential for the successful application of this technology. Various stem cell therapies are at different stages of development, with some already being used clinically, a few in preclinical trials, and some in the discovery stage.
Recommendations. Recent progresses suggest that stem cell therapy may have expanded clinical applicability in the future because they represent a viable therapeutic option for those who require tissue and cells replacement in diverse degenerative disease. More recently, major advances in the areas of stem cell biology, tissue engineering, and nuclear transfer techniques have made it possible to combine these technologies to create the comprehensive scientific field of regenerative medicine. "But there is a strong need for better understanding the biology, manipulation and safety of stem cells in tissue regeneration and repair before starting the therapeutic applications."

Review
PII: S225199391900009-9

Rec. 16 August 2018
Rev. 15 February 2019
Pub. 25 March 2019

Keywords
Embryonic Stem Cell, Mesenchymal Stem Cell, Regenerative

INTRODUCTION

Modern treatments for numerous degenerative diseases like Alzheimer disease, Parkinson disease, motor neuron disease, multiple sclerosis, diabetes, and kidney, liver, and heart diseases, as well as for several types of cancer, are mostly symptomatic, and for certain diseases, total recovery implies entire organ transplantation [1, 2]. Numerous applications of stem cells in tried and validated therapies are recognized in humans: starting from bone marrow transplants to more recent advances in skin and cornea repair [3]. Stem cell transplantation would probably have to be achieved within the window of time between the first appearance of injury and irreparable loss of neurons [4].

Up to date advancement shows that stem cell therapy that concerns cell reprogramming and transplantation of Embryonic Stem Cells (ESCs), Mesenchymal Stem Cells (MSCs) and induced pluripotent stem cells (iPSCs) represents an interesting so far disputed research area, with exciting results for many diseases [3, 5, 6]. Human iPS cell derivation previously required vectors that integrate into the genome, which can create mutations and limit the utility of the cells in both research and clinical applications [7].

The use of stem cells in the clinical field has gathered unbelievable momentum over the last decade, advanced by varying levels of achievement in clinical trials and by the advancement in our understanding of the mechanisms by which stem cells exert their seemingly favorable effects. Generally speaking, stem cells can be characterized as either embryonic or adult stem cells [8]. Stem and progenitor cells from adult tissues represent an important promise in the therapy of a number of pathological conditions [9].

Stem cell transplantation is being widely investigated as a potential therapy for cell death-related heart diseases [10]. This rapid translation into clinical studies has left a lot of questions concerning cell therapy

unanswered [11, 12]. There is rising evidence that stem cells secrete a variety of growth factors, cytokines, chemokines and bioactive lipids that control their biology in an autocrine or paracrine-manner and orchestrate interactions with the surrounding microenvironment [13].

The 21st century is witnessing an uprising in cellular therapy. Stem cell technology is proving to be a valuable tool not only for the development and regeneration of various tissue and organ systems, but also as a unit in evolution by natural selection [14]. Recently stem cell therapies are assumed to be used as safe and effective treatments. Even applications of stem cells are being investigated in clinical trials, including the use of stem cells to regenerate damaged tissues such as heart, skin, bone, spinal cord, liver, pancreas and cornea or to treat blood or solid-organ cancers [15].

So that stem cell research is a new field that is advancing at a hard to believe pace with new discoveries being reported from all over the world. Scientists have for years looked for ways to use stem cells to replace cells and tissues that are damaged or diseased. The miracles of stem cell application in incurable clinical conditions are being reported through media and newspapers [16]. To date, stem cell types which have been used in clinical trials include hematopoietic stem cells (HSCs), mesenchymal stem cells, neural stem cells, epidermal stem cells, endothelial progenitor cells, limbal stem cells, embryonic stem cells, and induced pluripotent stem cells [17]. The main properties that characterize stem cells include their indefinite capacity to renew themselves and leave their initial undifferentiated state to become cells of several lineages [18].

Heart failure (HF) is a leading cause of disability and death that accounts for approximately one million hospitalizations, over 50,000 deaths, and almost $35 billion in health care costs in the United States each year [19]. The use of stem cells in cardiology is frequently characterized as a matter of providing new myocytes, but it is much more complex than that. Whether global or segmental, heart failure is generally due to a specific cause, which must be removed as a precondition for the success of any reconstructive effort. Likewise, the mere generation of new vessels (by means of angiogenesis or vasculogenesis) [20]. Even more important, unlike the progenitor cells used in bone marrow transplants, the elementary myocardial functional units are not lone cardiomyocytes but, rather, are myocardial cells that are integrated into a multicellular assembly of myofibers. These cells are oriented in specific directions (indeed, implanted cell therapy should avoid generating myofiber disarray, which is a disease state in itself). Therefore, the challenges of stem cell treatment for the heart are much more complex than those of blood transfusion for anemia and bone marrow transplantation for bone marrow failure, which is the only clinically successful cellular treatments thus far [19].

Heart transplantation remains the ultimate approach to treating heart failure, but this is costly and excludes patients who are poor candidates for transplantation given their co-morbidities, or for whom a donor organ is unavailable. Stem cell therapy represents the first realistic strategy for reversing the effects of what has until now been considered terminal heart damage [21]. Therefore, in this review, We attempted to summarize the current status, available evidence, and present several clinical and nonclinical data concerning mainly the use of ESCs and MSCs in the treatment of different cardiovascular disease, highlighting both the opportunities and the limitations of stem cell therapy.

CURRENT STATUS OF STEM CELL

Embryonic Stem Cell

Since human embryonic stem cell (HESC) lines were first derived in 1998, these cells have been in high demand as objects of research. The ability of HESCs to reproduce almost limitlessly and to differentiate into many, if not all, cell types of the human body have generated an enormous amount of scientific interest. These unique capabilities provide a means of exploring many promising lines of research, which are likely to reveal a deeper understanding of human cellular biology and which may lead to potential cures for many diseases [22]. Embryonic stem (ES) cells are derived from totipotent cells of the early mammalian embryo and are capable of unlimited, undifferentiated proliferation in vitro. The term "ES cell" was introduced to distinguish these embryo-derived pluripotent cells from terato-carcinoma-derived pluripotent embryonal carcinoma (EC) cells [6].

Derivation of human embryonic stem cell (HESC)

HESC lines are conventionally derived from the inner cell mass (ICM) of pre-implantation stage blastocysts, of both good and poor quality, which have been donated for research and would otherwise be discarded. Morula-stage embryos or late-stage blastocysts (7-8 days) may also be used to create HESC lines. Although all the HESC lines derived worldwide share the expression of characteristic pluripotency markers

[23]. Many differences are emerging between lines that may be more associated with the wide range of culture conditions in current use than with the inherent genetic variations of the embryos from which HESC were derived [24].

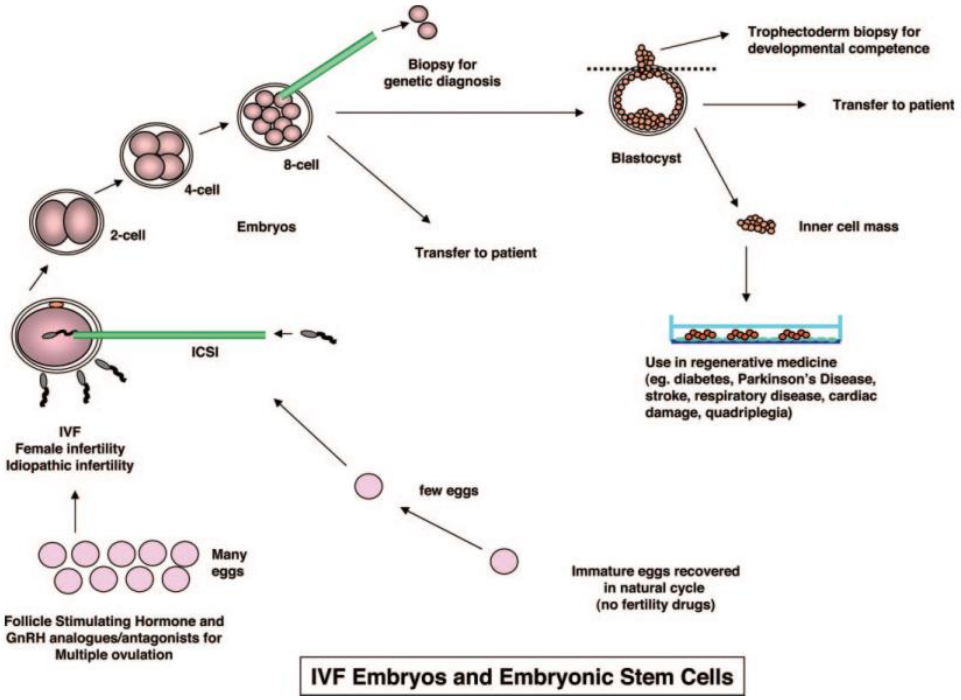

Figure 1. Derivation of HESC (human embryonic stem cell) [25].

Colonies of HESCs differ from the ICM in a number of ways. Firstly, ICM cells retain a memory for axes, dorsal-ventral, anterior-posterior, and left-right axes, that enables the differentiating cells to have position relationships that guide the differentiation, expansion, and integration of cell types required to form an organism. It is generally considered that ESCs are an epiblast derivative, or even a type of germ stem cell, that can be maintained as an immortal and pluripotential cell type under strict laboratory conditions, in the presence of secretory products of embryonic, or adult, somatic cells. Importantly, the self-renewal of HESCs appears to involve the Wnt family signaling pathway and probably other pathways that involve basic fibroblast growth factor (bFGF) and TGF-β [23].

In 1998, Thomson *et al.* [6] were as a first reporter of the successful derivation of HESCs from preimplantation human embryos. Their report followed they extensive studies by Thomson *et al.* [26] on the production of rhesus and marmoset ESCs. Intact blastocysts and mechanically isolated ICMs grown on mouse embryonic fibroblasts (STO cells) they are studied by the research group in Singapore from 1994–1996, and these cultures resulted in cell lines that differentiated after several passages *in vitro* [27].

The methods finally used successfully to establish HESC lines were described by Reubinoff *et al.* [28]. These methods were similar to those described by Thomson *et al.* [6, 29] and involved the isolation of ICM clusters from human blastocysts by immunosurgery and their co-culture with mitotically inactivated murine embryonic fibroblasts (MEFs). The HESCs form typical colonies of undifferentiated cells that need to be passaged weekly likely or, more often, as mechanically dissected colonies of 10 cells or more. Additional HESC lines have been derived by similar methods. More recently HESCs have also been derived under feeder-free conditions using cell-free lysates of MEFs [30].

The selection criteria used for choosing human embryos for deriving HESCs will determine the eventual success rates for their production. Small numbers of blastocyst-stage embryos grown in co-culture with human oviductal epithelial cells they are used by Reubinoff *et al.* [28] to produce six HESC lines after preliminary experiments involving around 30 embryos [23]. The six HESC lines they are derived from 12 blastocysts. This very high success rate of producing HESCs can be compared with the use of much larger numbers of embryos (blastocysts) by others. It is probable that about 50% of human embryos have chromosomal abnormalities, and it would be expected that these genetic errors would limit the success rate of HESC production. It is also difficult to establish HESCs from monosomic or trisomic embryos, with less than 10% made from human

embryos diagnosed as aneuploid. Interestingly, two HESC lines produced from trisomic embryos reverted to diploidy, indicating the embryos they are probably mosaic [31].

A large number of HESC lines have been produced from excess human IVF embryos by some IVF clinics; for example, Kukharenko *et al.* [32] reported 46 new HESC lines made from morulae, blastocysts, and ICMs isolated from blastocysts [33]. There was apparently little difference between stages of preimplantation human embryos in their capacity to form HESC lines. A more recent comparison of mechanical isolation of ICMs and plating whole blastocysts for deriving new HESC lines showed that mechanical isolation is more efficient. The use of antiserum raised in animals for immunosurgery to isolate ICMs is undesirable [34].

Genetic manipulation of human embryonic stem cell (HESCs)

Clonal derivation of HESCs is difficult, and the efficiency is extremely low [35]. However, it is possible to transfect HESCs with DNA constructs, and this is important for determining the role of transcription factors for the renewal and differentiation of HESCs. Identification of specific gene expression by reporter genes enables purification of cells of interest in differentiating cultures and the tracking of HESC derivatives in mixed cell cultures or when transplanted into animal models. Conventional transfection methods have been successful [36], as have lentiviral methods. Integration of reporter genes into controlling elements of specific genes or the approach of gene knock out or knock in used for functional genomics is very difficult because of the inability to clone HESCs. However, Zwaka and Thomson [37] have shown that it is possible to electroporate HESCs to achieve homologous recombination of HESC colony fragments. Gene function may be more appropriately determined in HESCs by using small inhibitory RNAs [38] to control renewal, differentiation, apoptosis and other mechanisms involved in cell function and response to internal and external stimuli.

Markers of human embryonic stem cell (HESCs)

Sperger *et al.* [39] have reported that, by microarray analysis, 330 genes are highly expressed in common in HESCs and human embryonal carcinoma cells and seminomas. This included *POU5F1* (*Oct4*) and *FLJ10713*, a homolog highly expressed in mESCs. Among those genes only highly expressed in HESCs and human embryonal carcinoma cells included a DNA methylase (*DNMT3B*), which functions in early embryogenesis, and *Foxd3*, a fork head family transcription factor that interacts with *Oct4*, which is essential for the maintenance of mouse primitive ectoderm [40]. *Sox2* is also highly expressed and is known to be important in pluripotentiality for example: The derivation of neural progenitor cells from human embryonic stem (ES) cells is of value both in the study of early human neurogenesis and in the creation of an unlimited source of donor cells for neural transplantation therapy. Here we report the generation of enriched and expandable preparations of proliferating neural progenitors from human ES cells. The neural progenitors could differentiate in vitro into the three neural lineages-astrocytes, oligodendrocytes, and mature neurons. When human neural progenitors were transplanted into the ventricles of newborn mouse brains, they incorporated in large numbers into the host brain parenchyma, demonstrated widespread distribution, and differentiated into progeny of the three neural lineages [41]. Embryonic stem (ES) cells are cells derived from the early embryo that can be propagated indefinitely in the primitive undifferentiated state while remaining pluripotent; they share these properties with embryonic germ (EG) cells. Serial analysis of gene expression (SAGE) has been reported by Richards *et al.* [42] and has been compare with some cancer SAGE libraries. As expected, *Oct4*, *Nanog*, and *Sox2* transcripts appear abundantly, but there were differences between HESCs in some other transcript abundance (*e.g.*, *Rex-1*).

Patient-Specific Stem Cells

There is much interest in the production of patient-specific stem cells using nuclear transfer techniques to introduce somatic cell nuclei into enucleated oocytes [23]. The reason for making HESCs for individual patients is for the possible establishment of immune-compatible cell derivatives for transplantation. It is important that new disease-specific stem cells be derived from patients with cancers; neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, motor neuron disease, and multiple sclerosis; and others of unknown cause or multigenic origins. The ability to reestablish pristine HESCs that can be differentiated in the laboratory to cells that will express the disease phenotype could be a very valuable resource for screening for molecules that interfere with the disease phenotype and identifying candidate drugs or molecular pathways that may enable a whole new approach to pharmaceuticals for these patients. This approach has already proven productive using mESCs [43].

Mesenchymal Stem Cells (MSCs)

Several progenitor cells can be found in human adult bone marrow. One class of multipotent adult progenitors is referred to as mesenchymal stem cells (MSCs). It is well documented that these cells are capable of differentiating into bone, cartilage, muscle, marrow stroma, tendon and ligament, fat, and a variety of other connective tissue [44]. Like the hematopoietic stem cells (HSCs) of marrow, the differentiation of MSCs involves multi-step cell lineages controlled by bioactive factors found in the local micro-environment or supplied in the culture environment of *ex vivo* cultivated cells. This controlled differentiation scheme was evolutionarily selected because it comprises a sequential process that can be modulated both in time and end-stage outcome; a multi-step pathway allows a large number of regulatory elements to be used to safeguard the final outcome [45]. Mesenchymal stem cells (MSCs), also referred to as connective tissue progenitor cells or multipotent mesenchymal stromal cells, have demonstrated significant potential for clinical use. Thus, MSCs have been the focus of a regime of emerging therapeutics to regenerate damaged tissue and treat inflammation resulting from cardiovascular disease and myocardial infarction (MI), brain and spinal cord injury, cartilage and bone injury, Crohn's disease, and graft-versus-host disease (GVHD) during bone marrow transplantation [46].

As part of the minimal criteria, human MSCs must adhere to tissue culture plastic; be positive for CD105, CD73, and CD90 and negative for CD45, CD34, CD14 or CD11b, CD79a, or CD19 and HLA-DR; and must be able to differentiate to osteoblasts, adipocytes, and chondroblasts under standard *in vitro* differentiating conditions [47].

Tissue sources of Mesenchymal Stem Cells (MSC)

The reported MSC frequency (as measured by CFU-F) and native concentration from several adult human tissues are reported. The relative abundance of MSCs throughout the body is understandable in light of recent findings that most, if not all, MSCs are of perivascular origin. Furthermore, there is a direct correlation between MSC frequency and blood vessel density in stromal vascularized tissue [48]. MSCs and pericytes share the phenotypic surface markers melanoma cell adhesion molecule (CD146) and platelet-derived growth factor receptor. It is hypothesized that pericytes are the in *vivo* source of MSCs, with cellular components protruding into the endothelial lumen of blood vessels to monitor and react to systemic signals. The widespread distribution of perivascular precursors for MSCs would account for their ability to respond to injury by sensing and secreting chemokines locally in response to injury, infection or disease in all vascularized tissues of the body [49].

Capacity of Mesenchymal Stem Cells (MSC)

Trophic properties of MSC: The primary trophic property of MSCs is the secretion of growth factors and other chemokines to induce cell proliferation and angiogenesis. MSCs express mitogenic proteins such as transforming growth factor-alpha (TGF-α), TGF-β, hepatocyte growth factor (HGF), epithelial growth factor (EGF), basic fibroblast growth factor (FGF-2) and insulin-like growth factor-1 (IGF-1) to increase fibroblast, epithelial and endothelial cell division. Vascular endothelial growth factor (VEGF), IGF-1, EGF, and angiopoietin-1 are released to recruit endothelial lineage cells and initiate vascularization [50].

Anti-inflammatory and immunomodulatory properties of MSC: MSCs hold up via paracrine mechanisms and change the regenerative environment via anti-inflammatory and immunomodulatory mechanisms. In response to inflammatory molecules such as interleukin-1 (IL-1), IL-2, IL-12, tumor necrosis factor-a (TNF-a) and interferon-gamma (INF-g), MSCs secrete an array of growth factors and anti-inflammatory proteins with complex feedback mechanisms among the many types of immune cells [49]. The key immunomodulatory cytokines include prostaglandin 2, TGF-b1, HGF, SDF-1, nitrous oxide, indoleamine 2, 3-dioxygenase, IL-4, IL-6, IL-10, IL-1 receptor antagonist and soluble tumor necrosis factor-a receptor. MSCs prevent proliferation and function of many inflammatory immune cells, including T cells, natural killer cells, B cells, monocytes, macrophages and dendritic cells [51].

Anti-apoptotic properties of MSC: In a situation where MSCs are administered with the aim of treating acute lesions, the first expected effect is the reduction of the extent of cell death, and this is observed in animal models of tissue injury and in co-culture experiments. Togel *et al*. reported that infused MSCs attach to the renal micro-vascular circulation and decrease apoptosis of adjacent cells in a model of acute kidney injury. In

order to elucidate the factors responsible for the observed renoprotective effect, these authors analyzed the MSC-conditioned medium and verified the presence of vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and insulin-like growth factor 1 (IGF-1), factors that enhance endothelial cell growth and survival [48]. Parekkadan *et al.* [52] found the presence of these and other anti-apoptotic molecules in MSC-conditioned medium and, interestingly, showed that an MSC-containing bioreactor connected to the bloodstream of rats experimentally subjected to fulminant hepatic failure resulted in the survival of 71% of the animals in contrast to 14% survival in the control group.

MSCs reduce apoptosis of UV-irradiated fibroblasts and lung epithelial tumor cells cultured under low pH and hypoxia, and the up-regulation and secretion of stanniocalcin-1 has been found to be at least partially responsible for this anti-apoptotic effect [53]. Also, adipose tissue-derived MSCs have been shown to express HGF, VEGF, transforming growth factor beta (TGF-β), basic fibroblast growth factor (bFGF, aka FGF2) and granulocyte–macrophage colony-stimulating factor (GM-CSF), and the expression of these molecules was found to increase under hypoxic culture conditions; particularly, VEGF upregulation under hypoxia has been shown to be greater than that observed for other factors [54].

Hypoxia takes place in the first stages of tissue injury, and secretion of anti-apoptotic factors by MSCs at this stage minimizes the extent of cell death in the tissues surrounding the injured areas; accordingly, in the latter study, it was further demonstrated that cultured, adipose-derived MSCs reduce necrosis and improve perfusion when injected into mice experimentally subjected to hind limb ischemia. Scientist's suggest that this anti-apoptotic activity could serve to limit the field of injury *in vivo* circumstances [54].

Table 1. Anti-inflammatory mechanisms of MSCs

Target Cell	Mechanism	Primery Effect	Secondary Effect
Dendritic cells	PGE2/direct contact	↓TNF-đ IL-12. differentiation and activation	↓Impairs effect on resting NK cells
Immature Dendritic cells	PGE2, IL-6. IL-8 and SDF-1 PGE2	↑IL-10	↓T.ceII proliferation
T cells (CD4 +, helper T cells)		↑IL-10	↓INF--, byTH1 cells'
T cells (CD8 +, Cytotoxic T-cells) Treg cells	IL-10, sHLAG5,IL-10	↓CD4 + T-cell proliferation by	↓rIL-4 by 1H2 ceilsa
		↓S-phase entry block and ↓Go/G1 phase arrest	↓ Treg production.
B-Cells	IL.10		IL-10 by Treg cells
	sHLAG5	↓Inhibits T-ceII functions	↓B-cell proliferation
NK-Cells	IL-10	↓InactivateT_H1- cells	↓Ig antibody production
		↓Cytotoxicity	↓by B cells
Monocytes	sHLA-G5	↑Treg Proliferation	
	PGE2. TGF- β 1, TGF-1, IDO. NO and PD-L1	↑IL-10 by Treg cells	
Macrophages	PGE2. IDO. HLA.G5. HGF. TGF- β 1	↓Trq differentiation	↓TNF-X and IL-1
Neutrophils	PGE2		
	IL-6		

Abbreviations: HGF, hepatocyte growth factor; HLA, human leukocyte antigen; IDO, indoleamine 2,3-dioxygenase; IL-1Ra, IL-1 receptor antagonist; INF, interferon; MMP, matrix metalloproteinase; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cell; NK, natural killer; NO, nitrous oxide; PD-L1, programmed cell death ligand-1; PGE2, prostaglandin 2; SDF-1, stromal cell-derived factor-1; sTNF-R, soluble TNF-a receptor; TGF, transforming growth factor; TNF, tumor necrosis factor; TSG, tumor necrosis alpha-stimulating gene; VEGF, vascular endothelial growth factor. A Promotes TH1-TH2 T-cell transition [46].

Antimicrobial properties of MSC: Assessment of direct inhibition of bacterial growth by MSCs or its conditioned medium (CM) was done by counting CFU. In brief, MSCs in 24-theyll plates (2×10^5 cells per well) in RPMI supplemented with 5% FBS were infected with 300 CFU *E. coli* or *S. aureus* and incubated for 6 hours in humidified CO_2 incubator, then aliquots of culture medium were taken from each well, serially diluted with

sterile PBS, and plated on LB-agar plates (Teknova, Hollister, CA). Colonies were counted after overnight incubation at 37°C. Antimicrobial activity of MSC CM (or synthetic LL-37) was tested by a Microdilution susceptibility test according to Andra *et al.* [55].

The researcher that studied human MSCs might express direct antimicrobial activity through the secretion of antimicrobial peptides. They examined the effect of human MSCs on bacterial growth *in vitro*. Expression of different antimicrobial peptides was investigated using reverse transcription polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), and immuno-histochemistry. Following stimulation with live *E. coli*, human MSCs produced one candidate antimicrobial peptide, LL-37, which was subsequently found to be responsible for antimicrobial activity in vitro. To determine if the secretion of LL-37 by MSCs would alter bacterial clearance in vivo, they tested BM-derived human MSCs in an *E. coli* pneumonia model in mice. Treatment with human MSCs, given 4 hours later, resulted in a significant reduction of *E.coli* colony-forming unit (CFU) in the lung homogenates (LHs) and the bronchoalveolar lavage (BAL) fluids. The effect was blocked with a neutralizing antibody to LL-37 demonstrating that human MSCs possessed antimicrobial activity, which is explained in part by the secretion of LL-37 [56].

Phenotypic characterization of Mesenchymal Stem Cells (MSC): After the discovery and early characterization of MSCs, scientists desired a method to prospectively isolate progenitor cells from bulk populations based upon positive or negative selection of CD markers expressed by the cells. The first markers unquestionably identified on MSCs were CD73 (SH-3/4) and CD105 (endoglin or SH-2), followed thereafter by CD90 (Thy- 1) and CD44. It since has been discovered that the quadruple-positive population of CD90þ/CD105þ/CD73þ/CD44 [57, 58]. It is common to fibroblasts and stromal cells, and only serves to discriminate these cell types from those of hematopoietic origin. Significant MSC phenotypic characterization has been published in the interim, but unfortunately there remains no strict consensus among the field [59]. In 2006, the International Society of Stem Cell Research established a minimum set of criteria for defining MSCs as: (1) plastic-adherent cells; (2) capable of tri-lineage (bone, cartilage and fat) differentiation; (3) phenotypically positive for CD105, CD73 and CD90; and (4) negative for CD45, CD34, CD11b, CD14, CD79a and HLA-DR [60]. However, these criteria are based on the characterization of *in vitro* cultured cells and do not apply to the native *in vivo* phenotype. For example, CD34 is considered a marker for hematopoietic stem cells and endothelial progenitors for freshly harvested cells in BM aspirate, but not MSCs [61].

Pericytes are stimulated by soluble growth factors and chemokines to become activated MSCs, which respond to the microenvironment by secreting trophic (mitogenic, angiogenic, anti-apoptotic or scar reduction), immunomodulatory or antimicrobial factors. After the microenvironment is re-established, MSCs return to their native pericyte state attached to blood vessels [55].

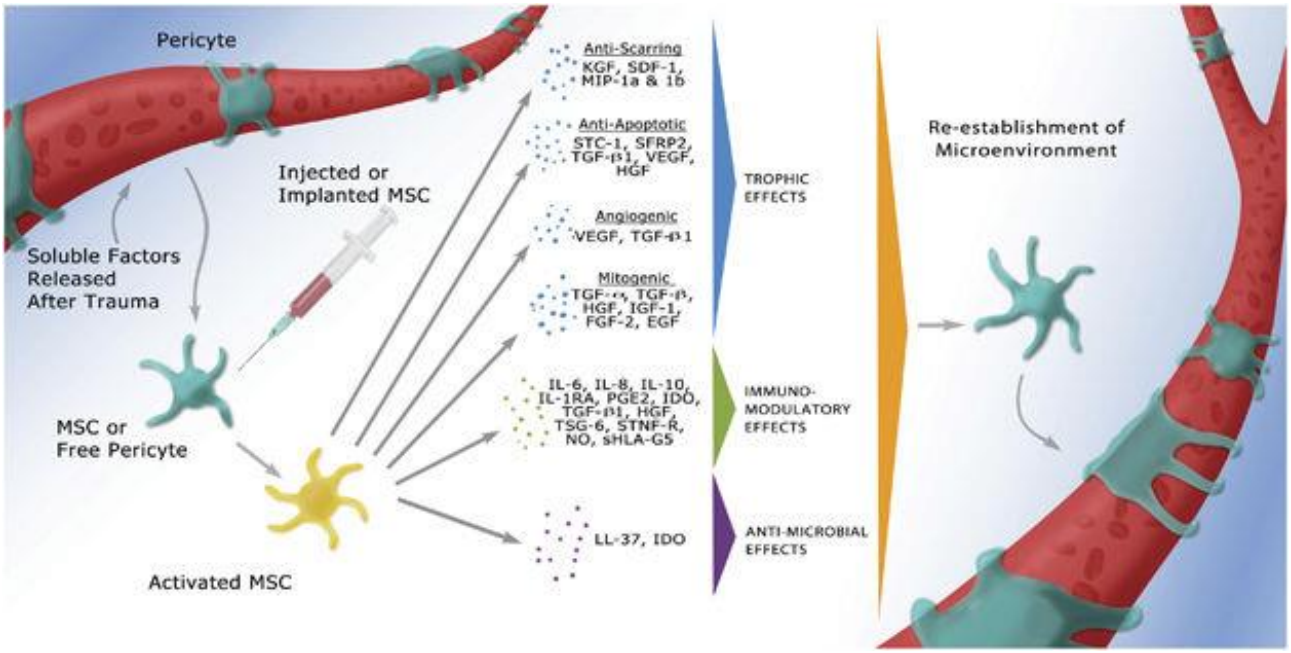

Figure 2. Phenotypic characterization of MSC.

CLINICAL TRANSLATION OF STEM CELL THERAPIES

Mesenchymal stem cells (MSC) in the treatment in cardiovascular therapies

Cardiac: Myocardial infarction is a multi-faceted insult to the cardiovascular system, stemming from the initial ischemic event; the extent of damage and subsequent cardiac disease correlates with the size of the original infarcted region [62, 63]. It is characterized by the disruption of blood supply to the heart muscle cells, which lead to myocardial infarction or death of cardiomyocytes. Reperfusion therapy or the restoration of blood flow by thrombolytic therapy, bypass surgery or percutaneous coronary intervention (PCI) is currently the mainstay of treatment for AMI and is responsible for the significant reduction in AMI mortalit. The efficacy of reperfusion therapy has led to increased survival of patients with severe AMI who would not otherwise survive. However, many (23%) of these survivors progress to fatal heart failure within 30 days. This phenomenon of an increasing number of severe AMI survivors contributes to an ever growing epidemic of heart failures [64].

Frantz *et al.* [63] have proposed the possibility of anti-inflammatory agents for minimization of deleterious post-myocardial infarction tissue remodeling. Several clinical studies have recently investigated the use of MSCs for this purpose; however, there has been no consensus yet on the preferred delivery method or type of cell. In a randomized, placebo-controlled study of chronic myocardial infarction patients receiving intra-myocardial injections of autologous BM-derived mononuclear cells, cell therapy patients had a decrease in summed stress score and increase in left-ventricular (LV) ejection fraction at 3 and 6 months (both statistically significant) [65, 66]. A subsequent study of 87 patients with severe LV dysfunction revealed no statistical differences in LV ejection fraction or size of infarct between placebo and autologous BMNC infusion [67]. A much smaller study revealed that both autologous BM MNCs and expanded BM MSCs yielded a decrease in myocardial scarring by 3 months, indicating beneficial tissue remodeling [68].

Similarly, the percutaneous stem cell injection delivery effects on neomyogenesis (POSEIDON) randomized trial comparing allogeneic and autologous MSCs in 30 ischemic cardiomyopathy patients indicated increased functional capacity, quality-of-life and ventricular remodeling as a result of both allogeneic and autologous cell therapy [69]. Most recently, direct myocardial injection of autologous, expanded BM MSCs resulted in persistent improvements in exercise capacity, Canadian cardiovascular scale (CCS) class score, angina attack frequency and nitroglycerin consumption at one-year post-intervention [70].

Opportunities and limitations of stem cell therapy

One of the limitations of applying cell-based regenerative medicine techniques toward organ replacement has been the inherent difficulty of growing specific cell types in large quantities [2]. Another obstacle that remains to be fully elucidated is the potential immune response to an ES and MSCs cell derived tissue graft and immune-mediated rejection, senescence-induced genetic instability or loss of function, and limited cell survival. This is demonstrated by the fact that nude mice, which lack T cells, are unable to mount a rejection response against an allogeneic skin graft. The unique ability of ES cells to give rise to HSC offers an interesting potential whereby immunological tolerance can be induced via hematopoietic chimerism [71].

Build out of regenerative service lines is predicated on effective clinical-grade biotherapies suitable for scale-up and standardized production and application. A viable supply chain requires quality-controlled manufacturing and delivery of products that fulfill patient specifications. Patient modifiers such as age, sex, morbidities, and concomitant therapies impact regenerative fitness. Cell performance is also subject to influences during procurement, production, and/or delivery. In fact, not all individuals harbor stem cells with a uniform reparative capacity [72].

CLINICAFUTURE PERSPECTIVES

The past decade has improved our knowledge of stem cell biology and the development of the cardiovascular system. However, a more profound understanding of cardiac myogenesis will be required for the development of advanced stem cell therapeutics to repair or regenerate damaged money disease [73]. The future will likely include (i) further investigation to delineate the human CM lineage tree; (ii) methods to isolate specifc cardiac progenitor pools or specialized CM subtypes; (iii) strategies to ensure survival of transplanted cells, their functional integration with the host myocardium, and circumvention of immune rejection; (iv) development of technologies to accurately assess integration; (v) determination of parameters that optimize engraftment, such

as delivery method, timing of transplantation post-MI, and cell preparations; and (vi) large-animal models of heart failure that closely resemble human cardiovascular physiology and disease for assessing cell engraftment, host immune response, and myocardial function [74].

Cell-replacement therapies hold great potential for treating Alzheimer's disease and related disorders patients. With the advent of stem cell technologies and the ability to turn stem cells into different types of CNS neurons and glial cells, some success in stem cell therapy has been made in animal models of Alzheimer's disease. Although these preclinical studies are promising, many more steps remain before stem cell therapies can be successfully used for the treatment of Alzheimer's disease and related disorders [75].

CONCLUSION

Stem cells therapy is under investigation for a number of therapeutic applications. These cells are known to home to some tissues, particularly when injured or under pathological conditions. The mechanisms underlying migration of MSCs and ESCs remain to be clarified, although evidence suggests that both chemokines and their receptors and adhesion molecules are involved. Different studies describe the role of chemokine receptors and adhesion molecules on stem cells may allow the development of therapeutic strategies to enhance the recruitment of *ex vivo*-cultured MSCs to damaged or diseased tissues. This could lead to various therapeutic possibilities such as supporting tissue regeneration, correcting inherited disorders (e.g., of bone), dampening chronic inflammation, and using these cells as vehicles for the delivery of biological agents. Further clinical data are necessary, however, to determine the *in vivo* distribution and therapeutic mechanisms of MSCs and ESCs to optimize their use as part of a personalized regenerative medicine strategy. This process will require the collaborative efforts of physicians, veterinarian, scientists, biotechnologiests, industry and regulatory agencies to translate nature's basic regenerative element into the continuum of clinical care. Stem cells are the potiential area for research and doing new regenerative engnering and cell therapy at the cell levels.

DECLARATIONS

Authors' contributions

MB conceived the review, coordinated the overall activity, and reviewed the manuscript. AK and MY supervising all in all activities.

Availability of data and materials

Data will be made available upon request of the primary author.

Acknowledgment

The authors' heartfelt thanks will also go to the university of the Gondar for resourse supporting.

Competing interests

The authors declare that they have no competing interests.

Consent to publish

Not applicable.

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