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*Review Article*

# Mesenchymal stem cell and regenerative medicine: regeneration versus immunomodulatory challenges

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**Abstract:** Mesenchymal Stem cells (MSC) are now presented with the opportunities of multifunctional therapeutic approaches. Several reports are in support of their self-renewal, capacity for multipotent differentiation, and immunomodulatory properties. They are unique to contribute to the regeneration of mesenchymal tissues such as bone, cartilage, muscle, ligament, tendon, and adipose. In addition to promising trials in regenerative medicine, such as in the treatment of major bone defects and myocardial infarction, MSC has shown a therapeutic effect other than direct hematopoiesis support in hematopoietic reconstruction. MSCs are identified by the expression of many molecules including CD105 (SH2) and CD73(SH3/4) and are negative for the hematopoietic markers CD34, CD45, and CD14. Manufacturing of MSC for clinical trials is also an important aspect as their differentiation, homing and Immunomodulatory properties may differ. Their suppressive effects on immune cells, including T cells, B cells, NK cells and DC cells, suggest MSCs as a novel therapy for GVHD and other autoimmune disorders. Since the cells by themselves are non-immunogenic, tissue matching between MSC donor and recipient is not essential and, MSC may be the first cell type able to be used as an "off-the-shelf" therapeutic product. Following a successful transplantation, the migration of MSC to the site of injury refers to the involvement of chemokines and chemokine receptors of respective specificity. It has been demonstrated that cultured MSCs have the ability to engraft into healthy as well as injured tissue and can differentiate into several cell types *in vivo*, which facilitates MSC to be an ideal tool for regenerative therapy in different disease types. However, some observations have raised questions about the limitations for proper use of MSC considering some critical factors that warn regular clinical use.

**Keywords:** Mesenchymal stem cell, MSC therapy, immunology, MSC application limitations

## Introduction

### *Basic considerations*

The existence of nonhematopoietic stem cells in bone marrow was first indicated by the German pathologist Cohnheim more than 100 years ago. His observations raised the possibility that bone marrow may be the source of fibroblasts that deposit collagen fibers as part of the normal process of wound repair [1]. Studies conducted in the early eighties demonstrated that the non-hematopoietic stromal cells within adult bone marrow, including reticular cells, smooth muscle cells, adipocytes and osteoblasts [2, 3] provide the local microenvironmental association necessary to support the survival, proliferation and differentiation of hematopoietic stem cells (HSC) [4]. Studies in rodents as well as humans identified a population of clonogenic marrow stromal cells, termed colony-forming unit fibroblasts (CFU-F), that were thought to be precursor cell populations capable of reconstituting all the cellular elements that comprise the supportive stromal tissue [5-7]. Further studies have supported the hypothesis that stromal populations are derived from multipotential bone marrow stromal cells (BMSC) or subsets of which are also referred to as bone marrow stromal stem cells (BMSSC) mesenchymal stem cells/marrow stromal cells (MSC) marrow-isolated adult multipotent inducible cells (MIAMI)] multipotent adult progenitor cells (MAPC) and mesenchymal adult stem cells (MASCS) [8-12].

Various tissues have been found to present MSC-like populations including adipose, muscle, tendon, dental pulp, periodontal ligament, umbilical cord blood, placenta, periosteum, liver, cartilage, synovium, synovial fluid, spleen, and thymus, using criteria established to describe bone marrow derived MSC [13-19]. However, variations in morphology, growth rates, proliferation potential and differentiation capacity have been reported in various tissue specific MSC-like populations. Nevertheless, they display many common characteristics attributed to their bone marrow counterparts, suggesting that MSC-like populations share a similar ontogeny. According to some, the perivascular niche is now thought to be a common stem cell microenvironment for resident MSC-like populations within the different tissues [8, 19-24]. Interestingly, various studies have noted a correlation between the location of MSC and the vasculature of their respective tissues of origin [20, 21, 25].

Phenotypically, MSCs express a number of markers, none of which, unfortunately, are specific to MSCs. It is generally agreed that adult human MSCs do not express the hematopoietic markers CD45, CD34, CD14, or CD11. They also do not express the costimulatory molecules CD80, CD86, or CD40 or the adhesion molecules CD31 (platelet/endothelial cell adhesion molecule [PECAM]-1), CD18 (leukocyte function-associated antigen-1 [LFA-1]), or CD56 (neuronal cell adhesion molecule-1), but they can express CD105 (SH2), CD73 (SH3/4), CD44, CD90 (Thy-1), CD71, and Stro-1 as well as the adhesion molecules CD106 (vascular cell adhesion molecule [VCAM]-1), CD166 (activated leukocyte cell adhesion molecule [ALCAM]), intercellular adhesion molecule (ICAM)-1, and CD29 [26-30]. There are several reports that describe the isolation of both human and rodent MSCs using antibody selection based on the phenotype of MSCs. Some have used a method of negative selection to enrich MSCs, whereby cells from the hematopoietic lineage are removed [31], others have used antibodies to positively select MSCs [32, 33].

MSCs from other species do not express all the same molecules as those on human cells; for example, although human and rat MSCs have been shown to be CD34 negative, some papers report variable expression of CD34 on murine MSCs [34].

The role of murine MSC on BM-Niche and subsequent HSC generation has been indicated through studies with P-alpha-S cells and Nestin + cells. These have shown important roles in the maintenance of the BM-perivascular and Endosteal Niche in terms of providing Niche related cells like adipocytes, Chondrocytes, resting reticular cells etc [35]. Recently, Nestin positive cells have become an attractive way to identify the mesenchymal cells. Nestin is an intermediate filament protein known as a marker for neuroepithelial stem cells. Its expression is transient and limited to early developmental stages as well as in various regenerating organs. However, the role of Nestin positive cells in murine hematopoietic niche is very important. It has been found that MSCs can be identified by Nestin expression which constitute an essential niche component. Furthermore, Nestin positive mesenchymal stem cells represent all the CFU-F forming activity and expanded with self renewal during propagation as non adherent mesensphere. Nestin+ cells colocalize with HSC and adrenergic nervefibre, and upregulate the HSC maintenance genes.These genes and others trigger osteoblastic differentiation and are selectively downregulated during HSC mobilization and β3 adrenoreceptor activation.Besides, it is documented that HSC home near Nestin+ cells and deletion of Nestin gene reduces HSC content in the bone marrow of mice. So, it is found that Nestin has an unprecedented role in murine hematopoietic niche [36].

It is generally accepted that all MSCs are devoid of the hematopoietic marker CD45 and the endothelial cell marker CD31. However, it is important to note that differences in cell surface expression of many markers may be influenced by factors secreted by accessory cells in the initial passages, and the in vitro expression of some markers by MSCs does not always correlate with their expression patterns in vivo [37].

However, there is also a variable expression of many of the markers mentioned due to variation in tissue source, the method of isolation and culture, and species differences [38]. Taken together, these examples illustrate that mesenchymal precursor cells are phenotypi-

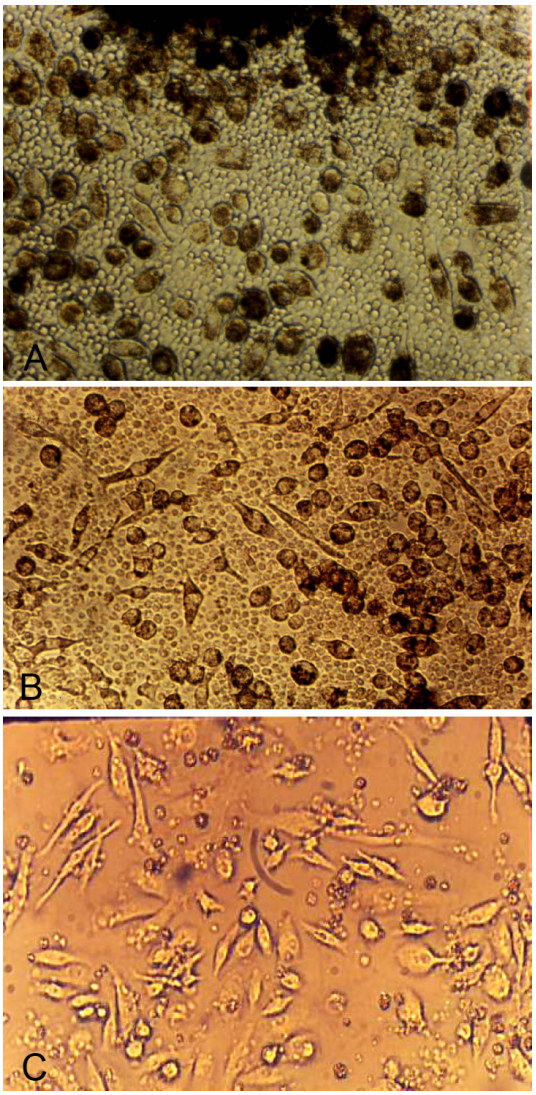


**Figure 1.** Enumeration of Bone Marrow derived adherent stromal cells /MSC with transforming characters: A. Whole bone marrow cells when cultured for more than 3 days in RPMI-1640 + 30% Fetal Bovine Serum (FBS) generated large stromal precursor cells with decaying HSCs and others. These precursor cells were tentatively transformed to stromal fibroblasts while HSCs were totally exhausted. B. The culture on subsequent days (after 9 days) represented significant transformation of the precursors into spindle shaped stromal fibroblasts with changes in morphology. The Generation of stromal fibroblasts from the precursors were in steady state. These cells were supposed to represent the bone marrow derived MSC. C. Elongated fibroblastic stromal appearance was apparent after 15 days of culture. The plate showed full confluence and considered to be matured stromal fibroblasts or Mesenchymal Stem cells (MSC).

cally heterogeneous, and the relationship between traditional bone marrow-derived MSCs and these other MSC-like populations remains to be fully clarified. Adult human MSCs are reported to express intermediate levels of major histocompatibility complex (MHC) class I but do not express human leukocyte antigen (HLA) class II antigens on the cell surface [39]. The expression of HLA class I on fetal hMSCs is lower [40] (**Figure 1A-C**).

### Clinical grade production of MSC

The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has proposed three criteria to define MSC; including ‘‘(1) the plastic adherence of the isolated cells in culture, (2) the expression of CD105, CD73 and CD90 in greater than 95% of the culture, and their lack of expression of markers including CD34, CD45, CD14 or CD11b, CD79a orCD19 and HLA-DR in greater than 95% of the culture, (3) the differentiation of the MSC into osteoblasts, adipocytes and chondroblasts in vitro’’. Whilst, these criteria is a common feature of different MSC populations, other important markers are often overlooked including NGF-R, PDGF-R, EGF-R, IGF-R, CD49a/CD29, STRO-1, STRO-3, CD146, or CD106, which have been shown to be efficient at isolating populations of human MSC with multi-lineage differentiation potential in vivo [8, 21, 41]. However, it must be noted that the constitutive expression of a handful of markers by MSC is not an indicator of homogeneity for any stem cell population. On the contrary, various markers that are used to purify MSC from marrow aspirates are rapidly down regulated following ex vivo expansion, correlating to an increase in gene expression associated with committed osteogenic cells [8]. Moreover, the heterogeneity of bulk MSC cultures is well illustrated in various studies that demonstrate the differential growth and developmental potentials exhibited by individually expanded MSC clones [8, 42-44]. As a consequence, researchers are actively attempting to determine the genotype and proteonomic profiles of long-lived multipotential MSC clones in order to elucidate the mechanisms that regulate and maintain primitive MSC and diseases such as osteoporosis, osteoarthritis, cancer and infection, the normal repair and remodeling processes are often impaired. Furthermore, other associated connective tissues such as cartilage, tendon and ligament demonstrate a

limited capacity for regeneration in response to damage caused by trauma or disease. For these reasons, different MSC preparations have been assessed as novel cell-based therapies to facilitate the developmental/remodeling processes required for the repair of damaged skeletal tissues, such as long bones, cranial bones, articular cartilage, ligament and tendons [45, 46].

Recently, the risk of transformation of MSCs during the culture process arose. It was demonstrated that adipose tissue derived MSCs can undergo spontaneous transformation after several months of culture [47]. The transformation process was shown by telomerase expression, karyotypic abnormalities, and tumor growth after injection into immunodeficient mice. These findings must drive to implement specific controls, the most rapid could be the telomerase expression checked by Q-PCR. Based on all these prerequisites, the Société Française de Greffe de Moelle et Thérapie Cellulaire (SFGM-TC) has developed a culture protocol of MSCs. The starting material is whole nucleated bone marrow cells. The culture medium consists of αMEM supplemented with 10% screened Stromal Vascular Fraction (SVF) and 1 ng/ml of Fibroblast Growth Factor2 (FGF2). The medium has changed twice weekly. At the confluence, the cells are passaged. Using CellStacks (Corning, USA) and specific connecting systems (Macopharma, France), all steps of culture process are done in close system. Starting from 60 millions of bone marrow nucleated cells, this process allows to obtain from 200 million up to 1 billion of pure MSCs in 28 days. The cultured cells show the phenotypic profile of MSCs, are multipotent differentiating through osteogenic, chondrogenic and adipocytic pathways, have immunosuppressive activity *in vitro*, and they are not transformed. The french national regulatory authority (AFSSaPS) gave the approval to produce MSCs for clinical study by using this method. Now, the SFGM-TC is starting a protocol to prevent by MSCs grafting the onset of GVHD.

Recent studies on isolation of MSC from both human and murine sources showed that the traditional plastic adherence technology of MSC isolation may interfere with the natural cell physiological character including differentiation and function (Immunomodulatory) and thereby render them relatively less therapeutic at a particular event [35]. They suggested prospected isolation of human MSC by flowcytometric identification of specific markers like CD49a, CD56, CD63, Cd73, CD105, Cd106, CD140b, CD271, MSCA-1, Stro-1, and SSEA4. CD146 has been considered to be the most important marker for Human Bone marrow derived MSC. The authors anticipated a more proliferative and therapeutically potential MSC with such method rather than the conventional one. Barry and Murphy identified the human MSC with the FACS and the clinicians can follow a new upgraded direction for MSC transplantation through the help FACS Aria [48].

## Immunological characteristics

### *Implanted cell-host interaction*

The question of the host response to implanted MSCs is critical and receiving attention as these cells are being considered in a variety of clinical applications. There are several aspects of the implanted cell-host interaction that needs to be addressed as we attempt to understand the mechanisms underlying stem cell therapies. These are (1) the host immune response to implanted cells, (2) the homing mechanisms that guide delivered cells to a site of injury and (3) differentiation of implanted cells under the influence of local signals.

### *Host immune response*

This topic has been the subject of some recent studies which have demonstrated that MSCs are capable of suppressing mixed lymphocyte reactions (MLRs) involving autologous or allogeneic T cells or dendritic cells. Di Nicola et al found that human T-cell proliferation, stimulated by the addition of irradiated allogeneic peripheral blood lymphocytes, dendritic cells or phytohaemaglutinin, was greatly suppressed when the cultures also contained MSCs [49]. They also found that this effect was reversed by the addition of monoclonal antibodies that had a neutralizing effect on TGF-1 and hepatocyte growth factor (HGF). This effect represents a specific suppression of MLR and is not due to apoptosis. Indeed a recent study by Kuroiwa et al shows that, in a murine model of allogeneic bone marrow transplantation, treatment with rhHGF strongly reduces the incidence of GVHD [50]. More recently, Tse et al. found that the

suppressive activity of MSCs on T-cell proliferation could not be accounted for by production of interleukin-10, TGF-1 or prostaglandin E2 [51]. Krampera et al. also suggest that MSCs inhibited both naive and memory T-cell responses and may function to physically hinder T-cell contact with antigen presenting cells in a non-cognate fashion [52]. Djouad et al. postulate that a soluble factor released by splenocyte-activated MSCs is involved in the immunosuppression and suggest that CD8+ regulatory cells are involved in the inhibition of allogeneic lymphocyte proliferation by MSCs [22].

The fact that Mesenchymal stem cells have immunomodulating properties and inhibit function of immune cells has been extensively discussed by many [52-62]. The specific molecular and cellular mechanisms involved in the immunoregulatory activity of MSCs are still under investigation and remain poorly understood. There is evidence that the capability to modulate immune responses rely on both cell contact-dependent mechanisms (i.e., through Jagged1-Notch1 interactions; Liotta et al., 2008) and paracrine effects through the release of soluble factors [63]. A broad panel of soluble factors have been involved including hepatocyte growth factor (HGF), prostaglandin-E2 (PGE2), transforming growth factor (TGF)-β1, indoleamine 2,3-dioxygenase (IDO), nitric oxide (NO), interleukin (IL)-10, heme oxygenase-1 (HO-1), and HLA-G5 [52, 55, 57, 59, 60, 64-67]. Differences in the mechanisms of immunomodulation employed by MSCs from different species have been reported. Whereas IDO activity appears to be a key player in human MSC-mediated immunomodulation, mouse MSCs do not express IDO and seem to use NO as the main mediator [64-66]. Interestingly, MSCs may also modulate immune responses through the generation of regulatory T cells [52, 54, 58, 70-71]. Whether this MSC-mediated Treg induction is due to an expansion of pre-existing Tregs, to a de novo induction or to a combination of both needs to be further explored.

Importantly, MSCs do not constitutively exert their immunomodulating properties but have to be "primed" by inflammatory mediators released from activated immune cells, such as IFNγ, IL1β, and TNFα [72, 73]. Also, the functionality of MSCs can be modulated by other inflammatory mediators such as APRIL and BAFF [74]. The thinking that MSCs are only anti-proliferative and immune-inhibitory on immune cells has been recently challenged by Waterman et al. who reported a "licensing" process of MSCs toward either anti-inflammatory or pro-inflammatory phenotypes, depending on the toll-like receptor (TLR) ligand used for activation [75]. The concept of MSC "licensing" has been discussed in the excellent review by Krampera [76]. In pursuance of the above, the immune phenotype of MSCs (widely described as MHC I-, MHC II-, CD40-, CD80-, CD86-) is regarded as nonimmunogenic and, therefore, transplantation into an allogeneic host may not require immunosuppression. MHC class I may activate T cells, but, with the absence of costimulatory molecules, a secondary signal would not engage, leaving the T cells anergic [77]. Many reports have also described MSCs as having immunosuppressive properties, specifically that MSCs can modulate many T-cell functions including cell activation [78, 79].

This suppression appears to be independent of MHC matching between the MSCs and the T cells. Some reports have demonstrated that direct cell-cell contact is required for suppression whereas others have shown that the suppressor activity depends on a soluble factor [80, 81]. It has also been shown that MSCs have immunomodulatory properties impairing maturation and function of dendritic cells and that hMSCs inhibit in vitro human B-cell proliferation, differentiation, and chemotaxis [82-85].

Despite some disagreement on the mechanisms by which MSCs exert their immunosuppressive effects, there is some evidence that these in vitro observations may translate to the in vivo setting. It has been reported that in vivo administration of baboon MSCs in immunocompetent outbred baboons significantly prolongs the survival of MHC-mismatched skin grafts [86]. Also, hMSCs have been administered in vivo to improve the outcome of allogeneic transplantation by promoting hematopoietic engraftment and to hamper graft-versus-host disease [87, 88]. In More recent studies, systemic administration of murine MSCs to mice affected by experimental autoimmune encephalomyelitis (a model of multiple sclerosis), a disease mediated by self-reactive T cells, resulted in a striking improvement in disease symptoms, mediated by the

induction of peripheral tolerance [89]. Therefore, targeting MSCs to inflamed tissues may have therapeutic benefit due to their immunosuppressive properties. However, another study investigated whether the immunosuppressive properties of murine MSCs could be of therapeutic value in the collagen-induced arthritis (CIA) mouse model (an established model of rheumatoid arthritis) to explore the effect of MSCs on disease progression [90].

Interestingly, they found that MSCs offered no benefit in the CIA model of arthritis; indeed, they found that MSCs were associated with accentuation of the Th1 response. Experiments in vitro showed that the addition of tumor necrosis factor alpha (TNF alpha) was sufficient to reverse the immunosuppressive effect of MSCs on T-cell proliferation, possibly accounting for the lack of improvement of the CIA. Hence, nonengineered MSCs may be unsuitable for the treatment of certain inflammatory diseases. Of course, these issues are central to the use of allogeneic MSCs in therapeutic applications.The use of allogeneic MSCs in therapeutic applications has many advantages, not the least of which is delivered in an acute setting, for instance following myocardial infarction. The disadvantage of an allogeneic approach relates to the potential risk of disease transmission from donor to recipient.

### Homing mechanisms and target specificity

The fact that MSCs can be differentiated into several different cell types in vitro, their relative ease of expansion in culture, and their immunologic characteristics clearly make MSCs and MSC-like cells a promising source of stem cells for tissue repair and gene therapy. However, compared with in vitro characterization, there is less information on the in vivo behavior of MSCs. The studies that have been performed can be split into observations following site-directed or systemic administration of cells.

Transplantation of human MSCs in fetal sheep resulted in long-term engraftment of the cells to various tissues, even after the development of immunocompetence [91]. It also seems clear that MSCs, when delivered by intravenous infusion, is capable of specific migration to a site of injury. This extraordinary ability of implanted cells to seek out the site of tissue damage has been demonstrated in the case of bone fracture, myocardial infarction and ischemic cerebral injury [92, 93]. In addition, MSCs, delivered as a free suspension by intra-articular injection in the knee joint following traumatic injury, are capable of specific engraftment to and repair of damaged meniscus and cartilage [94]. The mechanisms that guide homing of implanted cells are unclear, but in one study Wang et al showed that the chemokine monocyte chemoattractant protein-1 (MCP-1) in cerebral ischemic tissue promotes migration of infused MSCs to the site of injury [93]. They showed that MCP-1, not present in normal brain, is rapidly upregulated following middle cerebral artery occlusion in rats, and that it is chemotactic for MSCs. Homing and expansion of MSCs to the injured host was also elegantly demonstrated by Rombouts and Ploemacher [95]. They showed that in an irradiated host there were both migration and expansion of GFP-expressing syngeneic MSCs in the marrow and spleen. This was not the case with un-irradiated animals, again supporting the concept that these cells are specifically attracted to a wound environment. Interestingly, these authors also noted that the efficiency of homing of these cells was decreased following long-term culture, an effect that will influence the preparation of these cells for therapeutic use. The mechanism by which MSCs home to tissues and migrate across endothelium is not yet fully understood, but it is likely that injured tissue expresses specific receptors or ligands to facilitate trafficking, adhesion, and infiltration of MSCs to the site of injury, as is the case with recruitment of leukocytes to sites of inflammation. Chemokine receptors and their chemokine ligands are essential components involved in the migration of leukocytes into sites of inflammation, and it has recently been shown that MSCs also expresses some of these molecules. In addition, some of the adhesion molecules known to be involved in migration of leukocytes across the endothelium are also reported to be expressed on MSCs. It was found that hMSCs expressed functional (as determined by chemotaxis) CCR1, CCR7, CCR9, CXCR4, CXCR5, and CXCR6 on 43%-70% of cells. Another group reported expression of CCR2, CCR8, CXCR1, CXCR2, and CXCR3, as detected by real-time polymerase chain reaction and immunohistochemistry [96]. Ponte and colleagues demonstrated expression of

CCR2, CCR3, CCR4, and CXCR4 on hMSCs and found that TNF increased CCR2, CCR3, and CCR4 expression but not CXCR4 [97]. Thus, MSCs express a variety of chemokine receptors, although there is much variability among different report. A recent study by Ruster and colleagues [98]. suggested that P-selectin and a counterligand involved in the extravasation of hMSCs. These data suggest that hMSCs, like leukocytes, roll upon endothelial cells as the first stage in their recruitment. E-and L-selectins have been reported to be absent or present only in low amounts on hMSCs, and their significance in MSC trafficking, compared with P-selectin, may thus be unimportant [99-102]. Various integrin molecules, such as 1, 2, 3, 4, 5 are known to be expressed on hMSCs. Also, other adhesion molecules, which include VCAM-1, ICAM-1, ICAM-3, ALCAM, and endoglin/CD105, are expressed [103, 104]. So although it would seem likely that MSCs transmigrate into tissues by a similar mechanism to that of leukocytes employing some of the same molecules, specific differences in the use of adhesion molecules may also exist between these two cell types.

## Disease orientation and therapeutic success

There is another perspective on the role of adult stem cells in disease, and that is the concept that certain degenerative conditions, where there is progressive tissue damage and an inability to repair, may be due to the fact that stem cell populations are depleted or functionally altered. This has been considered in the case of osteoarthritis, a disease of the joints where there is progressive and irreversible loss of cartilage, with changes also in the underlying bone. In a study described by Murphy et al MSCs were prepared from marrow taken from patients with end-stage Osteoarthritis (OA) undergoing joint replacement surgery [105]. The marrow samples were harvested both from the site of surgery (either the hip or the knee) and also from the iliac crest. It was found that the proliferative capacity of the cells was substantially reduced in the osteoarthritic patients, and this was independent of the site of harvest. In addition, the chondrogenic and adipogenic activity of the cells was also significantly reduced, again independent of the site of marrow harvest. These effects were apparently disease-related, and not age-related, but additional studies will be necessary to confirm these preliminary observations. However, the data suggest that susceptibility to OA and perhaps other degenerative diseases may be due to the reduced mobilization or proliferation of stem cells. In addition, successfully recruited cells may have a limited capacity to differentiate, leading to defective tissue repair. Alternatively, the altered stem cell activity may be in response to the elevated levels of inflammatory cytokines seen in OA [106, 107].

### *Therapeutic applications*

Stem cell therapy involves the transplantation of autologous or allogeneic stem cells into patients, either through local delivery or systemic infusion. There is a precedent in haematopoietic stem cell transplantation, which has been used for some years in the treatment of leukemia and other cancers [108]. Some striking examples of the therapeutic use of marrow-derived MSCs have been reported recently. These addresses a broad spectrum of indications, including cardiovascular repair, treatment of lung fibrosis, spinal cord injury and bone and cartilage repair. Orlic et al. showed that locally delivered bone marrow cells can generate de novo myocardium, indicating that stem cell therapy can be useful in treating coronary artery disease [109]. Stamm et al. demonstrated the practical utility of this approach in a study involving the delivery of bone marrow cells into the infarct zone in patients following myocardial infarction [110]. The result of this treatment was a dramatic improvement in global heart function. Deb et al have also shown engraftment of bone marrow-derived cardiomyocytes in the adult heart following bone marrow transplantation [111]. Saito, Kuang, Bittira, Al-Khaldi, and Chiu demonstrated that MSCs are tolerated in a xenogeneic environment while retaining their ability to be recruited to the injured myocardium and undergo differentiation to a cardiac phenotype [112]. In vivo differentiation of MSCs to a skeletal muscle phenotype has also been demonstrated. Gussoni et al showed that murine MSCs, injected into the quadriceps muscle of *mdx* mice, expressed dystrophin in association with the muscle fiber sarcolemma, and pointed towards a potential therapy for muscular dystrophy [113]. Toma et al injected galactosidase-expressing human MSCs into the left ventricle of CB17 SCID/*beige*

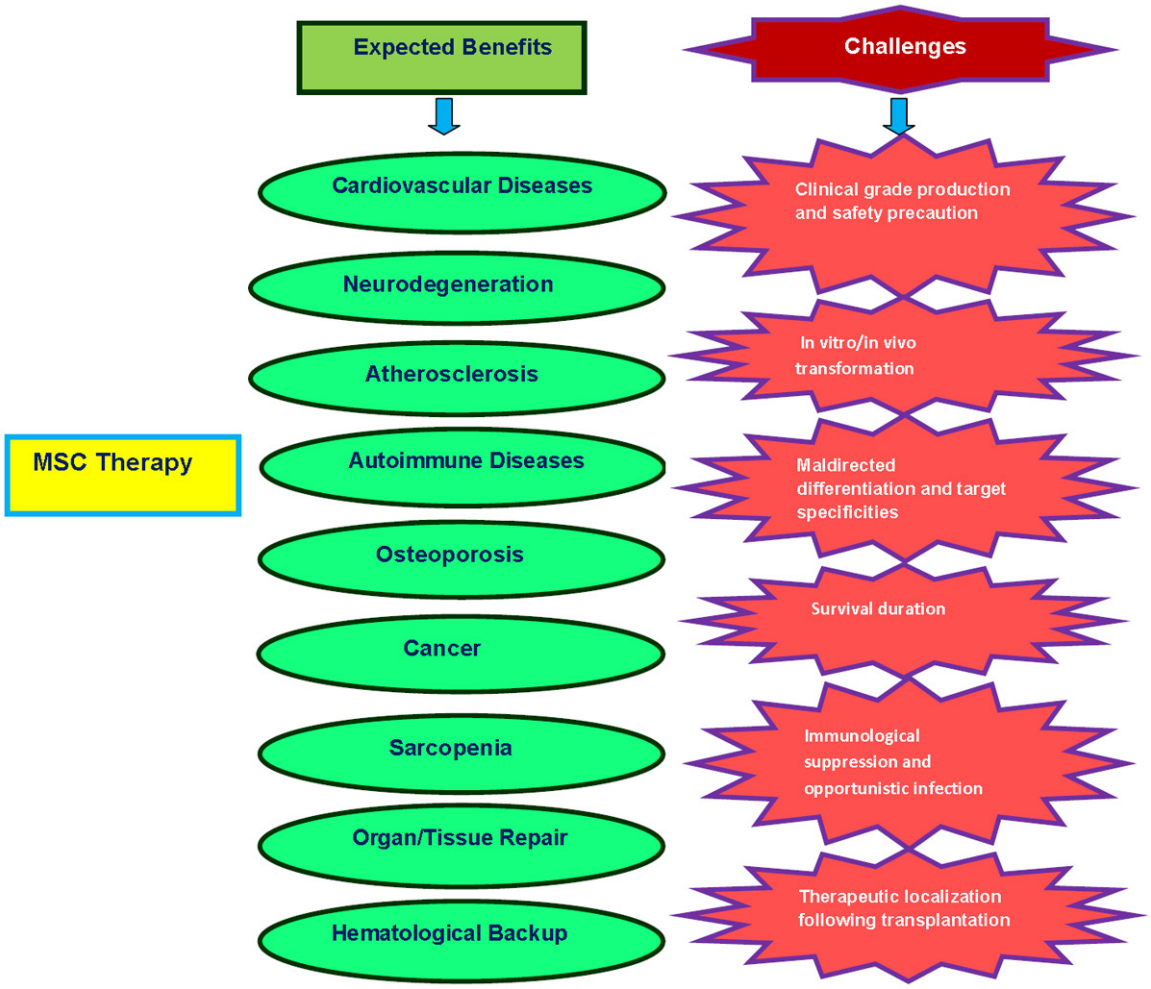


**Figure 2.** Schematics for MSC (Mesenchymal Stem Cell) therapy and the challenges: Therapeutic benefits of MSCs are varied but awaits full clinical success. The obstacles are required to be removed with proper monitoring and educating the cells with verified protocols.

adult mice, and found the labeled cells dispersed throughout the myocardium and expressing desmin, cardiac-specific troponin T, actinin and phospholamban, all indicative of differentiation of the engrafted cells to a mature myocardial phenotype [114]. MSCs have also been shown by Ortiz et al to engraft at high levels in lung tissue following exposure to bleomycin, and to offer protection against bleomycin-induced lung injury, including inflammation and collagen deposition [115]. These observations have broad implications in the area of lung disease associated with environmental damage. Stem cells with the ability to differentiate into neurons, astrocytes and oligodendrocytes have been isolated from rat spinal cord and implantation of neural stem cells in an adult rat model of spinal cord injury resulted in long-term functional improvement [116, 117]. Embryonic stem cells are capable of forming dopamine neurons in an animal model of Parkinson's Disease [118]. The ability of bone marrow-derived stem cells to differentiate into neural lineages in vitro and after transplantation in both mice and rats has been evaluated by Sanchez-Ramos leads to the conclusion that they may be useful in the treatment of stroke, traumatic injury and Parkinson's Disease [119]. Furthermore, it was recently demonstrated by Mezey that adult human bone marrow cells could enter the brain and generate neurons after transplantation [120]. These, and other equally dramatic observations underlie much of the current excitement and optimism about the use of stem cell therapy in the treatment of neuronal injury. In the area of orthopedic medicine there are also many examples of applications involving local delivery of marrow stem

cells. These include spine fusion, the repair of segmental bone defects and craniotomy defects [121-123]. Similar approaches have also been described in the repair of focal defects in articular cartilage and tendon [124-126]. In an animal model of osteoarthritis involving injury to the meniscus delivery of stem cells by intraarticular injection resulted in engraftment of those cells on the meniscus, fat pad and synovium with regeneration of meniscal tissue and protection of the cartilage [94]. The chondroprotective effects seen in these studies apparently derive from the regenerated meniscus since there is no evidence of direct engraftment of the implanted cells in the fibrillated cartilage. There is accumulating evidence of the hypoimmunogenic nature of MSCs and this has broad implications in terms of allogeneic therapy, or the delivery to a recipient of cells derived from an unmatched donor. There are several reports describing the clinical use of allogeneic donor-mismatched cells with little evidence of host immune rejection or GVHD. For example, allogeneic bone marrow transplantation in children with Osteogenesis Imperfecta resulted in engraftment of donor-derived MSCs and an increase in new bone formation [127]. Infusion of allogeneic MSCs in patients with Hurler's syndrome or metachromatic Leukodystrophy showed no evidence of alloreactive T cells and no incidence of graft-versus-host disease (GVHD) [128]. Engraftment of allogeneic MSCs has also been demonstrated in a patient with severe idiopathic aplastic anemia with improvement of marrow stromal function [129] (**Figure 2**).

*Limitations*

*In vivo differentiation:* The fundamental principle of stem cell therapy is that undifferentiated cells, following delivery to the injured host and migration to the site of injury, will, under the influence of local signals, differentiate into cells of the appropriate phenotype. These differentiated cells then contribute to the repair of the injured tissue. There is evidence to indicate that this is the case, but little or no data concerning the specific signals that give rise to differentiation in situ. For instance, cells implanted in an osseous defect, such as a large segmental gap in the femur, stimulate formation of new bone that can be assessed both radiologically and histologically [130, 131]. Similarly, Ponticiello et al showed that scaffolds loaded with MSCs and implanted in an osteochondral lesion on the medial femoral condyle give rise to both cartilage and bone cells. Several reports have also demonstrated that the delivery of murine MSCs to dystrophic *mdx* mice resulted in the implanted cells contributing dystrophin to the muscle fiber sarcolemma [124, 132]. It is therefore urged that before cell delivery the microenvironmental support should be assured in the host considering the disease abnormality.

*Immunological perspective:* Contradictory results have been reported that can be explained, at least in part, by the experimental conditions and the source of MSCs. The fact that differences in the experimental settings may lead MSCs to behave differently, suggests that MSCs can adjust their response in a dynamic way to the specific environmental conditions they face. In this regard, Waterman et al challenged the concept of MSCs being always immunosuppressive and suggested that a polarizing process toward a pro-inflammatory or anti-inflammatory phenotype may occur depending on the activity of Toll like Receptor (TLR) [75]. However, the anti-inflammatory and therapeutic effects reported in mouse models of sepsis and lung injury, where MSCs were exposed to high levels of LPS, seems to be in apparent contradiction to the polarizing process described in vitro. Therefore, the in vivo modulation of MSC biology by TLR ligands deserves to be further investigated and clarified.

The inflammatory conditions MSCs face when administered in vivo is now believed to play a fundamental role in their successful therapeutic use. Research on modulation of MSCs by TLRs can strongly contribute to better understand the immunomodulating properties of MSCs under different inflammatory environments and to characterize the features an inflammatory milieu should have for MSCs to best modulate immune reactions (i.e., composition, the ratio or activity of immune cells, cytokines or other inflammatory mediators such as TLR ligands).

*Tissue-specific stem cells:* Recent reports have provided substantial new insights into stem cell populations in a variety of adult tissues, raising new questions about tissue-specificity of MSC

like trabecular bone adipose tissue, synovium skeletal muscle lung and deciduous teeth [135-145]. In all cases the cells have been shown to differentiate along several defined pathways. For instance, De Bari et al showed that MSCs isolated from the synovium as an adherent cell population were capable of differentiation into chondrocytes, osteocytes and adipocytes. They also showed that these cells were capable of contributing to skeletal muscle regeneration in a nude mouse model and restored expression of dystrophin in the sarcolemma in dystrophic muscle of immunosuppressed *mdx* mice [142, 145]. Stem cells from adipose tissue, variously referred to as processed lipoaspirate (PLA) cells and adipose-derived adult stem (ADAS) cells have been shown to have similar differentiation potential [139-147]. De Ugarte et al. suggest that there is little difference between cells from marrow and fat in terms of yield, growth kinetics, cell senescence, multi-lineage differentiation capacity, and gene transduction efficiency [138]. The utility of these cells in therapeutic applications may then depend on the availability of tissue specimens and the ease of in vitro expansion. Henceforth, tissue specificity, cell source, their differentiation pathway all play important role in homing and successful clinical engraftment.

*Cell processing and safety precautions:* Mesenchymal Stem Cells (MSCs) are multipotent adult stem cells having an immunosuppressive effect. These characteristics lead to an increasing use of MSC in graft process or for regenerative medicine. For the clinical uses of MSCs, standards are needed. The clinical grade production necessitates adhering to good manufacturing practices (GMP) to insure the delivery of a "cell drug" that is safe, reproducible and efficient [148]. All parts of the process must be defined: the starting material (tissue origin, separation or enrichment procedures), cell density in culture, and medium (fetal calf serum (FCS) or human serum, cytokines with serum-free medium for the target). But to reach the GMP goal, cells have to be cultured in as close to a closed system as possible. Analytical methods are needed to assay the active compound and impurities. At a minimum, quality control (QC) of cells must consider the phenotype, functional potential, microbiological safety, and ensure the cultured cells remain untransformed. Finally, quality assurance system (QA) procedures specific to the production of MSCs as a cell drug must be determined and implemented [149].

## Concluding remarks

MSCs are under investigations 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 remain to be clarified, although evidence suggests that both chemokines and their receptors and adhesion molecules are involved. Studying the role of chemokine receptors and adhesion molecules on MSCs 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. Although early pre-clinical and clinical data demonstrate the safety and effectiveness of MSC therapy there are still many questions to be answered surrounding the mechanism of action. Additional information is required concerning the therapeutic efficacy of transplanted cells and the mechanisms of engraftment, homing and in vivo differentiation. There is also a need to carry out appropriately designed toxicology studies to demonstrate the long-term safety of these therapies. The widespread use of stem cell therapy will also depend upon the availability of validated methods for large-scale culture, storage and distribution. In addition, there is a need for novel engineered devices for tissue-specific delivery of cells, such as cell-coated stents and catheter-based delivery in cardiovascular applications, and arthroscopic delivery in the treatment of joint diseases. As these areas addressed new applications ,will be developed in future,leading to novel therapeutic opportunities. Much has been learned about stem cell therapy in the past few years, and much remains to be learned.

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### Disclosure of potential conflicts of interest

The Authors indicate no potential conflict of Interest.

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