

80788\_Auto\_Edited-check.docx

**Name of Journal:** *World Journal of Stem Cells*

**Manuscript NO:** 80788

**Manuscript Type:** REVIEW

**Mesenchymal stem cells in ischemic tissues regeneration**

Szydlak R. MSCs in ischemia

## **Abstract**

Diseases caused by ischemia are one of the leading causes of death in the world. Current therapies for treating acute myocardial infarction, ischemic stroke, and critical limb ischemia do not complete recovery. Mesenchymal stem cells (MSCs), whose immunomodulatory abilities and paracrine activity can ease inflammation and promote regeneration of damaged tissues, seem to be a hope and alternative therapy. This review aims to summarize the current knowledge on the mechanisms of action of MSCs and their therapeutic effects in the treatment of ischemic diseases.

**Key Words:** Ischemia; Mesenchymal stem cells; Regenerative medicine

Szydlak R. Mesenchymal stem cells in ischemic tissues regeneration. *World J Stem Cells* 2022; In press

**Core Tip:** Mesenchymal stem cells (MSCs) transplantation is an innovative therapy for many ischemic diseases with positive therapeutic effects. Ischemia of an area is defined as insufficient blood supply to specific tissues and various organs or individual parts of the body. The leading cause of tissue ischemia is the narrowing or blockage of the artery's lumen, most often due to the formation of atherosclerotic plaques, thrombus, or spasms of a specific artery. Here, the potential therapeutic mechanisms of MSCs in ischemic diseases are discussed, along with examples of preclinical and clinical studies.

## **INTRODUCTION**

Ischemia of an area is defined as insufficient blood supply to specific tissues and various organs or individual parts of the body (*e.g.*, limbs). Reduced blood perfusion causes inadequate transport of oxygen and nutrients to tissue-resident cells, leading to ischemia and ultimately damaging tissues and organs. The leading cause of tissue ischemia is the narrowing or blockage of the artery's lumen, most often due to the formation of atherosclerotic plaques, thrombus, or spasms of a specific artery<sup>[1]</sup>.

Disruption of blood flow to particular organs or parts of the body may be chronic developing over several months or years, or acute - occurs suddenly (*e.g.*, during exercise) and usually takes a more rapid course, and its consequences often have a worse prognosis. Ischemia resulting from atherosclerotic lesions is most commonly found in the heart muscle, lower extremities, kidney, and the brain<sup>[1-4]</sup>. Examples of ischemic diseases caused by the narrowing of the coronary and cerebral arteries that cause high morbidity and mortality in patients are myocardial infarction and ischemic stroke (Figure 1)<sup>[5]</sup>. Ischemia may entail several functional changes at the level of individual cells that build tissues, causing their dysfunction and death due to necrosis or apoptosis<sup>[6]</sup>.

Standard treatments for ischemia include invasive and pharmacological control, and treatments for the effects of ischemia in the damaged tissues. These methods emphasize improving the quality of and extending the patient's life, but they cannot fully reverse the effects of tissue ischemia<sup>[7-11]</sup>. Even with the substantial progress of therapeutic strategies, these approaches do not provide the expected clinical benefits in all patients. Therefore, novel treatment pathways while replacing or supporting classic therapeutic approaches should continue to test<sup>[12-14]</sup>.

Currently, great hopes are placed in regenerative treatment, *i.e.*, therapies based on cellular preparations, including using various progenitor and stem cells (SCs) and their products<sup>[8,12,15]</sup>. Over the past decade, growing afford has been directed to the regenerative properties of SCs in relation to the biological treatment strategy of substitution of damaged cells in the tissue with new ones<sup>[16]</sup>. It is also believed that SCs may be involved in the neovascularization of ischemic tissues<sup>[17,18]</sup>.

In 1970, Friedenstein *et al*<sup>[19]</sup> discovered exceptional type of cells that has been extensively researched over the years for its potential use in regenerative medicine to treat ischemic damage<sup>[20]</sup>. These cells, called mesenchymal SCs (MSCs), reside in both young and adult donors [*e.g.*, umbilical cord, amniotic fluid, umbilical cord blood (UCB), placenta, adipose tissue (AT), bone marrow (BM), dental pulp (DP), and others] which has been a particular and exciting source of SCs for many years, mainly for

autotransplantation and allotransplantation. They now account for the most popular used population of SCs in clinical trials worldwide (clinicaltrials.gov). MSCs derived from birth-related tissues may be more hopeful due to better proliferative capacity compared with MSCs obtained from adult tissues. This is because they are safe in terms of both sourcing and ethical aspects<sup>[21,22]</sup>. Due to several specific characteristics, these cells are essential candidates for regenerative therapies for ischemic tissues. Moreover, it is possible to use these cells to manufacture ready-to-use SC-based medicinal products. This review describes the potential therapeutic mechanisms of MSCs in the context of ischemic disorders treatment. Exemplary clinical trials and procedures enhancing the therapeutic effect of MSCs also are discussed.

### **PATHOGENESIS OF ISCHEMIC DISEASES AND SC-BASED THERAPY**

The leading cause of ischemic disorders is the chronic inflammatory disease of the arteries, called “atherosclerosis”. It can be caused by many risk factors, including ageing, high blood pressure, diabetes, hypercholesterolemia, and smoking<sup>[23,24]</sup>. In the course of atherosclerosis, pathological damage and dysregulation of the endothelium lining the blood vessel wall, accumulation of lipids, smooth muscle cells, leukocytes, and foam cells are observed. Also, there is aggregation of platelets in the lumen of the blood vessels, which leads to the formation of plaques narrowing the lumen of the vessels<sup>[25,26]</sup>. Moreover, in atherosclerotic lesions, increased expression of matrix metalloproteinases and their participation in weakening the vascular wall through the degradation of the extracellular matrix have been demonstrated<sup>[27]</sup>. To compensate for the degradation of extracellular matrix (ECM), smooth muscle cells migrate from the outer layers of the artery wall to the sheath’s inner lining to increase the collagen secretion rate<sup>[24]</sup>. Although, it often causes undesirable remodeling as macrophages secrete cytokines such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , and IL-6 to induce smooth muscle cell apoptosis<sup>[24]</sup>. The unbalanced degradation rate of the extracellular matrix caused by increased collagen production results in the formation of atherosclerotic plaques with a thin fibrous collagen cap<sup>[28]</sup>. Injecting MSCs at this stage

can modulate immune cell function, metalloproteinase (MMP) activity, and the secretion of proinflammatory cytokines, and restore collagen homeostasis<sup>[29]</sup>. In addition, due to hemodynamic changes and high shearing stresses in atherosclerotic plaques, ruptures and bleeding may occur, increasing platelet recruitment, coagulation processes, and thrombus formation<sup>[30]</sup>. Due to the secretion of pro-angiogenic factors and the ability to differentiate in the endothelium, MSCs can promote angiogenesis to restore blood flow to ischemic tissues for tissue regeneration and organ function restoration<sup>[31]</sup>.

### **MSCS - KEY PLAYERS IN ISCHEMIC TISSUES REGENERATION**

Over the past decade, scientists and clinicians often discuss the regenerative properties of SCs in the context of biological treatment approaches. These strategies involve the replacement of damaged tissue cells with new SCs, including ischemic myocardium<sup>[16,32]</sup>. SCs are also considered cells that can participate in the neovascularization of ischemic tissues, which may also be associated with the improvement the function of this organ<sup>[18,33,34]</sup>.

One type of cell studied for years for their potential future use in regenerative medicine to treat myocardial damage or improve perfusion in limb muscle tissue is the mesenchymal/stromal SC (MSC) isolated from the BM, AT, birth-associated tissues, and other sites. These cells, was found in both young and adults donors, and have been an essential and exciting source of SCs, primarily for autotransplantation. They now account the most often used SC population in clinical trials worldwide (clinicaltrial.gov)<sup>[18,35,36]</sup>.

The history of studies on MSCs began in the 1970s when Friendenstein *et al*<sup>[19]</sup> observed colony-forming units-fibroblast (CFU-F) (today known as MSCs). These cells constituted a fraction of adherent cells in the BM<sup>[19]</sup>. Moreover, their previous studies showed that subpopulations of BM cells could differentiate towards, *i.e.*, osteoblasts<sup>[37]</sup>. These discoveries initiated an increase in interest in MSCs and the search for these cells in other tissues as well.

One of the primary and best-known sources of MSCs is BM<sup>[38,39]</sup>. Cells with similar morphology and biological characteristics can also be found in other tissues collected from adult donors, such as peripheral blood, AT, DP, and fetal tissues such as UCB and cord Wharton jelly (WJ)<sup>[40]</sup>.

BM-MSCs show morphological and phenotypic similarities to AT-MSCs and WJ-MSCs. These markers include CD29 +, CD44 +, CD73 +, CD90 +, CD105 +, CD166 +, human leukocyte antigen (HLA)-ABC +, CD11b-, CD14-, CD19a-, CD34-, CD45-, CD79-, and HLA-DR-<sup>[41,42]</sup>. However, the source tissues from which MSCs are isolated differ in the content of these SCs. For example, the number of MSCs in the BM is lower than in AT<sup>[43,44]</sup>. The BM contains from 0.0017% to 0.02% of MSCs among all mononuclear cells, while in AT, these cells constitute even 5.0% to 25.6% of all cells, the so-called stromal-vascular fraction obtained from this tissue<sup>[43,45]</sup>. The number of CFU-F colonies formed after sowing the same number of cells isolated from BM or AT is several times higher than AT about BM<sup>[41]</sup>. Also, WJ-MSCs cells have a higher frequency of CFU-F colony formation than BM-MSCs cells<sup>[46]</sup>. Considering the differentiation potential of BM-MSCs, AT-MSCs, and UC-MSCs, these cells show a comparable capacity to differentiate into osteoblasts, chondrocytes, and adipocytes<sup>[41,47]</sup>, confirming their mesodermal origin.

MSCs derived from various tissues can also be differentiated *in vitro* into phenotypically similar cells, including cardiomyocytes (CMCs), vascular endothelial cells (ECs), and nerve cells. However, it has been shown that the effectiveness of such differentiation is variable and depends on the tissue origin<sup>[48-55]</sup>. Within one culture of MSCs, cells may be more or less predisposed to differentiate to a specific phenotype of the mature cell<sup>[56]</sup>. Despite the morphological and antigenic similarity between MSCs from different source tissues, the results of world studies show that BM-MSCs may offer a different expression profile of many genes compared to placenta-derived MSCs<sup>[56]</sup>, which suggests that MSCs obtained from BM, WJ, and AT may differ in terms of their molecular composition and their ability to differentiate.



The International Society for Cellular Therapy, to clarify the nomenclature and define the requirements of human MSCs, and facilitate the comparison of test results between laboratories around the world, proposed three minimum criteria for characterizing human MSCs<sup>[57]</sup>.

Due to numerous world studies showing the relatively wide potential of MSCs to differentiate into various types of tissues, these cells - constituting a population obtained from mature tissues, have for years interested scientists in the context of their potential use in tissue regeneration<sup>[58-60]</sup>.

### **MSCS MECHANISMS OF ISCHEMIC TISSUE REGENERATION**

Multipotent MSCs show many features desirable from the point of view of their potential use in the regeneration of damaged tissues - not only in autologous but also in allogeneic transplants, therefore, over the last decade, many studies have been undertaken to explain the mechanisms of action of these cells replaced by tissue damage. Figure 2 presents several essential mechanisms of action contributing to ischemic tissue regeneration.

#### ***Differentiation of MSCs***

Initially suggest that MSCs administrated into the area of tissue damage are able to differentiate into desired cell type, including muscle-building cardiac cells, *i.e.*, CMCs, vascular smooth muscle cells (VSMCs), and ECs<sup>[59,61,62]</sup>. Shown *in vivo* that MSCs injected to the heart muscle have a phenotype similar to differentiating cells, including CMCs and ECs<sup>[63-65]</sup>. Pochon *et al*<sup>[66]</sup> confirmed that WJ-MSCs differentiated into CMCs *in vitro*, expressing CMCs markers and spontaneous throbbing, which might evidence that their terminal maturation into specialized cells capable of playing their proper functions. Although MSCs show the potential to differentiate towards CMCs *in vitro*, the fundamental issue is to restore damaged tissue *in vivo*. Effective delivery and retention must be emphasized here because if cells do not reach the target tissue, they cannot exert any therapeutic effect<sup>[60]</sup>. Even if studies have shown that MSCs can



differentiate into various types of myocardial cells, an increasing number of studies show that this is not a main mechanism for their regenerative activity in cardiovascular system.

Several articles now contain information on the neural differentiation of MSCs from various sources *in vitro*<sup>[67-69]</sup>. In the described studies, the prevailing view suggests that MSCs, derived from immature tissues due to their plastic properties, can differentiate more effectively into cells with neural phenotypes, showing the presence of typical linear markers<sup>[70]</sup>. Positive results of *in vitro* neural differentiation of MSCs were obtained in experiments using specific chemical compounds, growth factors, co-culturing with mature neurons, or the culture of three-dimensional aggregates<sup>[71,72]</sup>. Another study showed that neural differentiation of MSCs can be induced by a conditioned medium derived from cultures of olfactory ensheathing cells or Schwann cells<sup>[73]</sup>. Sotthibundhu *et al*<sup>[74]</sup> discovered that stimulation of autophagy MSCs could improve the efficiency of SC differentiation and the formation of neural-like cells.

#### ***Interaction of MSCs with other cells***

MSCs can communicate with neighboring cells through direct cell-cell interactions, including gap junctions and tunneling nanotubes<sup>[75]</sup>. Moreover, MSCs are able to <sup>1</sup>transport mitochondria through nanotubes and thus obtain cardiological protection by regaining the respiratory chain in myocytes<sup>[76]</sup>. To allow damaged tissue to regenerate, MSCs communicate with other cells in the damaged areas to recruit other types of SCs. For example, studies have demonstrated that CMCs can re-enter the cell cycle after supplementation with specific cytokines secreted by MSCs [*e.g.*, transforming growth factor (TGF)- $\beta$ ]<sup>[77]</sup>. MSCs can also keep other cells to active migration to the area of tissue damage, as demonstrated by the trafficking of hematopoietic SCs to the damaged myocardium<sup>[78]</sup>.

#### ***Immunomodulatory properties of MSCs***

Despite the reported low rate of retention of MSCs in ischemic heart muscle after their injection, the results many experiments showed improvement in the functional heart parameters, like inhibition of adverse tissue remodeling myocardium and left ventricle ejection fraction (LVEF)<sup>[32]</sup>. So, this begs the question: Is the improvement in injured organ function following the administration of MSCs results only from the implantation and ability of MSCs to differentiate into specific cell types, or another mechanism is also involved in this process?

No doubt, that in order to answer this question, other studies, which have been focused on the immunomodulatory properties of MSCs should be mentioned here<sup>[79-81]</sup>. The remarkable ability of MSCs to produce an enormous number of soluble factors, like anti-inflammatory cytokines enables them to modulate the immune system response<sup>[82,83]</sup>. For example, MSCs secrete the cytokines IL-4 and IL-10 which inhibit the proliferation of T cells, the growth factor hepatocyte growth factor which inhibits the proliferation of CD4 + T cells, and TGF- $\beta$ 1 which, with prostaglandin E2 inhibit the inflammation process<sup>[84-86]</sup>. Moreover, they encouraged the maturation of monocytes towards anti-inflammatory macrophages type M2<sup>[79,87]</sup>.

In addition, the most extraordinary attribute of MSCs is an immunological privilege. MSCs are known to be capable of avoiding and suppressing immune responses<sup>[88,89]</sup>. Most MSCs show the low expression of HLA class I and lack of HLA class II markers. Due to this feature, they do not cause an immune conflict between the transplant recipient and the injected cells. Additionally, MSCs possess the HLA-G, which is a key factor in elimination of the fetus rejection by the maternal immune system<sup>[90,91]</sup>. Because of high expression of HLA-G, MSCs can modulate the tolerance of immune system and it has a very beneficial effect on acceptance of the transplant.

### *Paracrine effects of MSCs*

Another reported mechanism of therapeutic activity of MSCs can be attributed to secretion paracrine factors, including several cytokines, growth factors, and chemokines, that may regulate many regenerative processes at the MSCs implantation

site<sup>[92]</sup>. Pro-angiogenic molecules produced by MSCs involve, among others, the protein fibroblast growth factor-2 (FGF-2)<sup>[92-94]</sup>, platelet-derived growth factor<sup>[95]</sup>, and an extremely pro-angiogenic vascular endothelial growth factor, supporting the proliferation of VSMCs and ECs as well as the migration and the new blood vessel structures formation<sup>[96]</sup>. On the other hand, molecules that promote ECM remodeling involve MMP1, MMP2, MMP9, a family of enzymes that degrade ECM structure, and TNF- $\alpha$  and the activator plasminogen which leads to ECM proteins impairment<sup>[78,97]</sup>. A distinct category of molecules produced by MSCs are the factors molecules responsible for MSCs survival, proliferation, and migration to the area of tissue injury, which involve FGF-2 supporting the proliferation of VSMCs and ECs, stromal cell-derived factor-1 reducing apoptosis and regulating cell migration, insulin-like growth factor-1 controlling cell differentiation, growth and inhibiting apoptosis, a secreted frizzled-related protein-2 supporting CMC survival at the conditions of low oxygen availability *in vivo*<sup>[77,98]</sup>.

### ***Exosomes secretion***

Extracellular vesicles (EVs) are biological nanoparticle structures containing bioactive molecules, including protein, and nucleic acids. They can influence other cells and participate in intercellular communication over long distances<sup>[99]</sup>. Many studies on tissue regeneration mechanisms demonstrated that EVs released by SCs can deliver bioactive molecules to target cells, which may influence the function of those cells, including the process of damaged tissue regeneration<sup>[100]</sup>.

### **PRECLINICAL STUDIES: ANIMAL MODELS OF TISSUE ISCHEMIA**

Most of the molecular and cellular mechanisms, which affect the therapeutic potential of MSCs in the therapy of ischemic tissues were initially identified in animal models. The capacity of MSCs to survive in the recipient's body after administration and the ability to differentiate into ECs and CMCs has been proven in acute myocardial infarction in a mini-swine model<sup>[65]</sup>. In this paper, Zhang *et al*<sup>[65]</sup> also confirmed the

migratory activity of MSCs towards inflammation, inhibition of the CMC's apoptosis, stimulation of cardiac SCs, reduction of fibrosis, myocardium reverse remodeling, and enhancement of the LVEF. It has been demonstrated that MSCs derived from UCB (UCB-MSCs) can reduce the AMI size by  $\geq 50\%$  and enhance LVEF<sup>[101]</sup>. The observed therapeutic effect may be due to the ability of MSCs to secrete bioactive factors. In turn, thanks to their immunosuppressive properties and the paracrine effect, MSCs can alleviate inflammation and ischemic heart disorders, contributing to the reduction of infarct size and improving LVEF through a paracrine effect<sup>[102]</sup>. The therapeutic efficacy of UC-MSCs and heart function improvement<sup>[103]</sup>. Intravenous administration of MSCs has improved LVEF contractility, function, perfusion, and reverse remodeling<sup>[18]</sup>. The transplantation of MSCs in a rat model of acute myocarditis can reduce inflammation by decreasing the infiltration of an inflammatory cell, reducing CMC death, and adverse myocardium remodeling<sup>[104,105]</sup>. Based on the results of animal studies, hopes are high regarding enhancing many heart functions, such as reduction of scar tissue, myocardium reverse remodeling, increase in cardiac contractility, improvement of ejection fraction, and increase in heart perfusion. However, there is still a need for long-term observation of the effects of injecting MSCs to ensure the safety and efficacy of therapy.

In preclinical studies on transgenic animals that are models of neurological diseases, significant functional improvement was observed after MSC cell transplantation<sup>[106]</sup>. The use of these cells may be related to their direct action, *i.e.*, replacement of damaged cells as a result of neural differentiation, or to an indirect influence positively influencing the endogenous regenerative processes of the organism. In addition, preclinical studies on animals have confirmed the neuroprotective properties of MSCs transplants, which may be linked to their <sup>5</sup> production of numerous growth, anti-inflammatory, and anti-apoptotic factors important for neurons<sup>[107]</sup>.

#### **CLINICAL TRIALS: MSCS AS MODERN THERAPEUTIC AGENTS IN ISCHEMIA**

Clinical trials testing MSCs in regenerative therapy are growing (ClinicalTrials.gov). Regenerative therapies based on MSCs for several ischemic disorders are now carefully examined. The remedial effects obtained are promising and prove that the transplantation of MSCs may be beneficial in the treatment of many diseases<sup>[108,109]</sup>. The concept of clinical application of MSCs can give huge hope to improve the health of patients suffering from many cardiovascular diseases<sup>[110]</sup>. So far, a few dozen studies have been registered in the international database of clinical trials to assess the safety and effectiveness of MSCs administration in the treatment of ischemic diseases.

The most popular source of therapeutic MSCs used in clinical practice is BM. The first MSC-based biological drug - AMI HeartiCellgram® used in myocardial infarction therapy was also based on BM-MSCs. Lee *et al*<sup>[111]</sup> described the manufacturing procedure for this drug, and Kim *et al*<sup>[112]</sup> proved its effectiveness in a clinical experiment. The therapeutic benefits included the restoration of normal systolic heart function and the reduction of post-infarction scar tissue<sup>[113,114]</sup>.

The pioneer pilot clinical study applying WJ-MSCs in treating ischemic disorders was performed by Musialek *et al*<sup>[115]</sup> in 2015. This group has shown the safety of injected MSCs as an off-the-shelf product, percutaneous allogeneic SC therapy in human acute myocardial infarction. Later observation proved no clinical adverse events in the treated tissue, except for a local rise in body temperature of one patient<sup>[115]</sup>. Currently, Musialek *et al*<sup>[115]</sup> are examining the safety and effectiveness of the “CardioCell” drug (WJ-MSC-based biological therapeutic) in a phase II/III randomized, placebo-controlled, double-blind clinical trial in several ischemic disorders (*i.e.*, acute myocardial infarction (EudraCT Number: 2016-004662-25), chronic ischemic heart failure (EudraCT Number: 2016-004683-19), and non-option critical limb ischemia (EudraCT Number: 2016-004684-40)<sup>[116,117]</sup>.

A clinical trial with the MSCs has proved the enhancement in heart muscle function in cases of heart failure. For example, Bartolucci *et al*<sup>[118]</sup> have shown that intravenous administration of UC-MSCs improved LVEF, functional status, and standard of living. Also, exosomes released by UC-MSCs can alleviate the effect of acute myocardial



ischemic injury<sup>[119]</sup>. Scientists confirmed that the injection of UC-MSCs exosomes can greatly improve contractile heart function and minimize myocardial fibrosis. These bioactive bubbles protected heart cells from death and supported EC migration and angiogenesis. UC-MSCs have also been applied in a clinical study for the treatment of chronic coronary occlusion<sup>[17]</sup>. Also in this trial, the improvement the heart function and better left ventricular ejection fraction were reported<sup>[17]</sup>.

So far, several clinical trials of ischemic stroke have shown that transplanting MSCs into patients with successful reperfusion therapy reduces the volume of lesions after stroke and promotes the regeneration of neurological function. This success is shown by improvements in human functional, behavioral and sensorimotor assessments, such as the Barthel Index, Modified Rankin Scale, European Stroke Scale, Fugl-Meyer Scale, and National Institutes of Health Stroke Scale<sup>[120,121]</sup>. MSCs participate in the regeneration of ischemic tissues and organs with beneficial effects, as outlined above. The therapeutic activity is presumed to include immunomodulation, cardioprotective effects, activation of endogenous repair processes, and tissue remodeling.

### **HOW TO IMPROVE THE EFFECTIVENESS OF MSC-BASED THERAPY?**

MSCs can supply alternative therapy in the treatment of a lot of disorders, but many studies have demonstrated that depending on the obtained method of isolation, expansion, and delivery we can obtain cells with distinct functional features. The therapeutic benefits of MSC-based therapy involve paracrine activity, immunomodulation, and enhanced function of the damaged organ. However, not all patient's responses to treatment are satisfactory, therefore this approach requires a deep understanding of the therapeutic actions of MSCs after injection into the recipient's organism. The therapeutic efficacy of MSCs is affected by many factors, including the method of MSC cultivation *in vitro*, the metabolic activity of the MSCs, the number of injected cells, the patient's genetic sensitivity, and the stage of the disease<sup>[122,123]</sup>.

The selection of the appropriate source of therapeutic MSCs depending on the disease is crucial as more and more data show source-dependent variations in therapeutic

activity such as levels of released trophic proteins or different differentiation capacities. There is much disagreement as to the therapeutic efficiency of MSCs derived from different tissues (fetal and adult sources). Therefore, extensive studies are desirable to obtain consistent data about remedial effects.

Another problem that is much debated is the type of transplant, *i.e.*, allogeneic or autologous. The results obtained from clinical trials showed no difference between the therapeutic effects of allogeneic and autologous MSCs in the treatment of ischemic cardiomyopathy<sup>[124]</sup>. The undoubted advantage of autologous transplants is the lack of burdening the cells with other diseases because in such cases the donors are healthy volunteers. Autologous SCs obtained from the patient do not have this privilege, which may limit the therapeutic effectiveness. In addition, it is an important issue to obtain the right dose of autologous cells, which takes time the autologous transplant requires the collection of appropriate tissue from the patient, isolation of SCs, and obtaining the necessary dose of therapeutic cells, which is a challenge as the disease and the patient's age may contribute to the reduction of the proliferative activity of MSCs<sup>[125,126]</sup>.

To resolve these issues, scientists introduced allogeneic sources of SCs which can be used to produce ready-to-use biological products. The use of allogeneic SCs shortens the waiting time for a transplant. Allogeneic cells from young and healthy donors are used to produce biological drugs of quality that can be stored frozen. The medicinal product prepared in this way, if necessary for clinical intervention, can be thawed at any time and administrated to the patient.

MSCs applied in the clinic as therapeutic agents must be carefully prepared, according to the good manufacturing practice (GMP) and good clinical practice (GCP) standards, with the established quality control system. The manufacturing process should be properly optimized in terms of therapy requirements to reach a sufficient remedial effect. MSCs applied in the clinic as therapeutic agents must be carefully prepared, according to the GMP and GCP standards, with the established quality control system. The manufacturing process should be properly optimized in terms of therapy requirements to reach a sufficient remedial effect. Here it should be



emphasized that we can prepare therapeutic cells to treat different disorders, such as graft *vs* host disease, myocardial infarction, Crohn's disease, and others, however, the therapeutic benefits obtained may not be satisfactory. Therefore, there is still a need for a more sophisticated approach to obtaining highly effective biological medicines. The use of an appropriate approach to the production of therapeutic MSCs for the treatment of a particular disease should contribute to the achievement of satisfactory results.

Additionally, to adopt therapeutic MSCs for demanding conditions in the host body, preconditioning methods can be applied. This approach requires the presence of additional adverse factors (such as low oxygen availability or proinflammatory cytokines) during *in vitro* culture. Bernardo and Fibbe<sup>[127]</sup> during the production of therapeutic MSCs, added to the culture medium pro-inflammatory cytokines, the concentration of which increases after acute myocardial infarction. In this study, an anti-inflammatory response of MSCs was observed within 24-48 h based on the analysis of the composition of the culture medium. The use of selected cytokines in MSCs cultures for the treatment of acute myocardial infarction enhanced anti-inflammatory secretory activity and therapeutic efficacy.

## CONCLUSION

The remarkable ability of MSCs to regenerate damaged body parts to regain lost function raises great hopes for clinical practice in many disorders, including ischemic diseases. Three properties of MSCs render them optimal for ischemic tissue repair and regeneration: (1) Immunomodulatory and immunoregulatory capacity beneficial to ameliorate abnormal immune responses; (2) Soluble and insoluble paracrine factor-generating potential; and (3) Endothelial differentiation.

While MSCs have several advantages, there are still many challenges to overcome. The unique immunomodulatory properties of MSCs are essential to their function, but the mechanisms of the immune regulation of MSCs have not been elucidated. Many factors can influence the therapeutic potential of MSCs, such as donor age, isolation and culture method, induction factors, oxygen concentrations, mechanical stimuli, and

others. Hence, optimizing the culture conditions of MSCs may be an effective way to improve the therapeutic potential of MSCs for successful tissue repair.

# 9%

SIMILARITY INDEX

### PRIMARY SOURCES

- |       |   |                 |
|-------|---|-----------------|
| 1     | "Stem cells: From Potential to Promise", Springer Science and Business Media LLC, 2021<br><small>Crossref</small>   | 150 words — 3%  |
| <hr/> |   |                 |
| 2     | www.hindawi.com<br><small>Internet</small>  | 123 words — 3%  |
| <hr/> |   |                 |
| 3     | www.ncbi.nlm.nih.gov<br><small>Internet</small>   | 94 words — 2%   |
| <hr/> |   |                 |
| 4     | Marta Bryk, Elżbieta Karnas, Jakub Mlost, Ewa Zuba - Surma, Katarzyna Starowicz. "Mesenchymal stem cells and extracellular vesicles for the treatment of pain: Current status and perspectives", British Journal of Pharmacology, 2021<br><small>Crossref</small> | 28 words — 1%   |
| <hr/> |   |                 |
| 5     | Renata Szydla. "Chapter 23-1 Advances, Opportunities, and Challenges in Stem Cell-Based Therapy", Springer Science and Business Media LLC, 2022<br><small>Crossref</small>  | 13 words — < 1% |
| <hr/> |   |                 |
| 6     | pure.uva.nl<br><small>Internet</small>  | 13 words — < 1% |
| <hr/> |   |                 |
| 7     | docplayer.net<br><small>Internet</small>  | 12 words — < 1% |

---

EXCLUDE QUOTES            ON

EXCLUDE BIBLIOGRAPHY   ON

EXCLUDE SOURCES           < 12 WORDS

EXCLUDE MATCHES           < 12 WORDS