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Preconditioning and remodeling of mesenchymal stem cells (MSCs) for enhanced wound healing potential and cellular therapy: A priming tool in Regenerative Medicine

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Abstract

Cellular therapies are highly effective for protecting and repairing tissues damaged by injury or chronic illness within the field of regenerative medicine. Hypoxia, or low oxygen conditions, can impact both normal and disease processes. While hypoxia can sometimes result in cell death and brain damage, it can also initiate an adaptation process known as hypoxic preconditioning. This process is essential for cell and tissue survival and is considered a key endogenous defense mechanism, offering a promising approach for treating ischemic injuries. Research has identified various cellular mechanisms through which hypoxic preconditioning exerts its protective effects. This preconditioning significantly boosts the resilience, regenerative capabilities, and healing potential of cells when transplanted into a host environment, thereby enhancing therapeutic outcomes in numerous disease models. Research has re-demonstrated that forming remedies can stimulate stem cells, enhancing both enhancing the formation of new neurons and the development of new blood vessels in brain. By integrating cell-based therapies with treatments that provide neuroprotection, facilitate healing, reduce inflammation, and support rehabilitation, the therapeutic benefits can be significantly augmented. This review delves into recent advancements in the varieties of cells utilized and their roles in regenerative medicine, also potential upcoming therapies and treatments.

Key words: Regenerative medicine, transplantation therapy, Stem Cells, Hypoxic Preconditioning, Mesenchymal

Introduction & Background:

Stem cells, no matter their origin, can be used in various therapies, possess the ability for self-renewal and multi-lineage differentiation, potentially developing into an entire adult organism. These properties drive the rapid advancement of regenerative medicine [1]. Nonetheless, there are instances where the host's body rejects the transplanted cells. The creation of adult induced pluripotent stem cells (iPSCs) enables their use in various autologous and allogenic disease conditions, establishing them as one of the furthermost valuable and stimulating cell sources in stem cells treatment [2,3]**.** Stem cells are generally categorized into three primary types: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells such as mesenchymal stem cells (MSCs). ESCs are derived from the inner cellular mass of initial-stage embryos; these cells exhibit a high proliferation rate, can divide indefinitely without showing signs of aging, and possess multi-lineage differentiation capabilities. Literature has indicated that various transcription factors, such as OCT4 and NANOG, are crucial for sustaining the highest pluripotency condition of ESCs **Fig 1** [4].

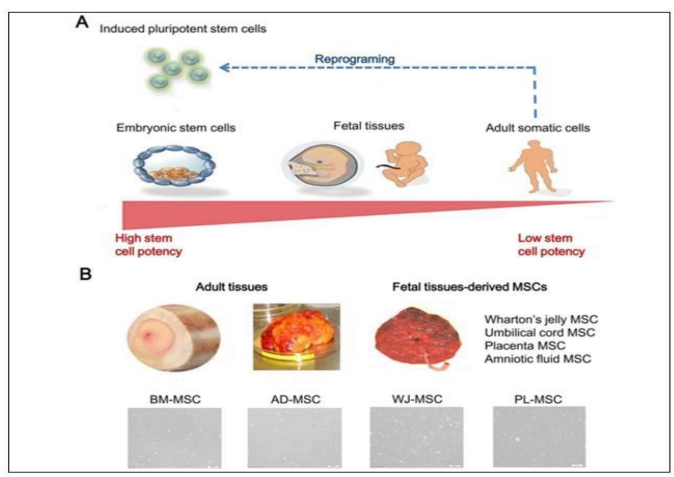

Figure 1: Types of stem cells

Subsequently, iPSCs were generated by reprogramming somatic cells into embryonic stem cells undifferentiated condition through the expression of numerous key regulatory proteins [5]. Even though both categories of stem cells exhibit high pluripotency, unlimited self-renewal, and multi-lineage capabilities, their use requires careful consideration due to potential ethical and safety concerns, as well as low efficacy. Conversely, mesenchymal stem cells (MSCs), which are plentiful and present in almost all tissues, constitute a type of adult stem cell with multi-lineage potential [6,7]**.** Furthermore, there are no ethical issues associated with using these cells for various clinical conditions and treatments. They are also regarded as an ideal origin for cell-based therapy to preserve homeostasis in both normal and disease conditions. Additionally, their low risk of immunological rejection permits their use in various autologous and allogeneic transplantation scenarios [8,9,10]. These cells have a spindle-shaped morphology and exhibit plastic adherence in in vitro culture conditions. They can proliferate extensively and differentiate into various cell types, such as adipocytes, osteocytes, chondrocytes, hepatocytes, endothelial cells, cardiomyocytes, and neural cells. Furthermore, studies have indicated that these cells can potentially differentiate into cardiac or neural cells at low rates after being infused into animals. However, MSCs primarily promote regeneration in damaged organs through various paracrine mechanisms [9,10].

Types of Stem cells

In vivo, MSCs typically exist in a diverse micro-environment with less oxygen stress, around one or five percent oxygen, whereas the oxygen level in a standard culture environment (20-21% O2) is significantly higher than their natural living conditions [11]. This alteration in oxygen tension in vitro can reduce cellular functions, such as proliferation, differentiation, anti-inflammatory properties, healing potential and the ability to repair damaged and dysfunctional organs and tissues. Literature indicates that cells isolated from unhealthy individuals or those cultured for extended periods exhibit decreased self-renewal potential and compromised physiological conditions. Additionally, inflammation in damaged tissues or organs leads to the apoptosis or senescence of transplanted MSCs due to the production of reactive oxygen species (ROS) and disruption of antioxidant mechanisms and healing potential [12,13]. Wound healing is a crucial and dynamic process that involves three primary stages: inflammation, proliferation, and tissue remodeling. During the inflammatory phase, cell migration, cytokines, and growth factors act as key inflammatory mediators, influencing vascular proliferation and subsequently tissue remodeling. The therapeutic potential of stem cells in wound regeneration has been extensively researched. Various types of stem cells possess distinct characteristics that contribute to wound healing. In recent time, the function of mesenchymal stem cells in healing mechanisms has recognized, also, significant progress has been made in studies focusing on MSCs. Numerous treatments have established to address long-lasting wounds. obsolete treatments have preferred for removing dead tissue, using wound coverings, delivering antibiotics, and carrying out skin graft procedures as needed. Newly developed treatments involve biophysical methods such as electrical stimulus and shock wave therapy to expedite wound healing [13,14,15]**.** Despite considerable advancements in stem cells therapy for skin wound repair, the full potential of stem cells has to come realized. Implanted stem cells often have a limited lifespan and low survival rates at the wound site. Consequently, one objective in optimizing cell therapy is to enhance cell survival. Additionally, another goal line is to indorse cellular properties [16].

Recent studies have demonstrated that preconditioning strategies, genetic modifications, and optimization of the culture environment are crucial approaches to enhancing the functionality of MSCs both in vitro and in vivo. These modifications can improve the potency and efficacy of MSC transplantation, and improve wound healing potential, making them a valuable tool in tissue engineering and regenerative medicine for various clinical conditions [16,17]. Pretreated MSCs show improved cell survival, enhanced multi-lineage differentiation, greater paracrine effects, and better homing abilities to injury sites. Preconditioning MSCs with low oxygen levels, pharmaceutical compounds, chemical substances, growth factors, signaling molecules, and mechanical factors enhances the survival signaling and enables them to withstand the severe and harsh microenvironments encountered during MSC transplantation **(Fig 2).** In this study, we review current approaches to optimize and enhance the wound healing effectiveness of stem cell [16,17,18].

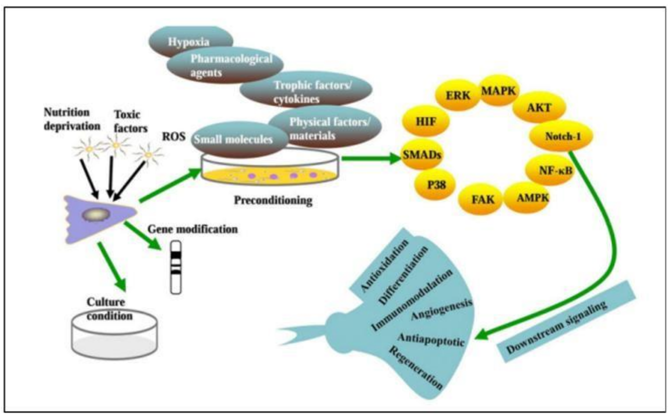

Fig. 2: Preconditioning strategies aim to enhance the role of MSCs both in vitro and in vivo by activating several critical signaling pathways, thereby protecting tissues and organs from damage and injury [16]**.**

Hypoxic preconditioning: enhance the cellular characteristics and wound healing capacity of MSCs:

In vitro experiments demonstrated that subjecting MSCs to hypoxic conditioning for 15 minutes at 2.5% O2, followed by 30 minutes of reoxygenation at 21% O2, and subsequent 72-hour hypoxic conditioning at 2.5% O2 significantly enhances proliferation and migration rates [18]. This regimen also enhances the countenance of numerous pro-existence genes and multiple regulatory proteins in MSCs. Furthermore, it augments the multi-lineage potential of MSCs in in vitro settings. Several investigations have explored the advantageous effects of hypoxia or hypoxia/reoxygenation (H/R) in optimizing oxygen concentrations to enhance healing potential, cellular processes and activities, as well as the therapeutic potential of MSCs [19,20]. One study demonstrated that exposing adipose-derived MSCs to hypoxic conditions at 0.5% O2 for 24 hours significantly enhances their ability to differentiate into multiple lineages and healing potential, acting as a protective factor. Interestingly, another in vitro study indicated that Preconditioning MSCs with 3% oxygen diminishes the occurrence of aneuploidy relative to standard oxygen levels at 20% O2, suggesting that Low-oxygen preconditioning enhances genetic and chromosomal integrity, thereby enhancing the resilience and health of MSCs [20,21]. Studies on rats with traumatic brain injury have shown that preconditioning with 0.5% oxygen enhances the secretion of multiple growth factors, including hepatocyte regulatory proteins (HGF) and VEGF. This improvement contributes to enhanced movement and mental abilities and healing activity across various animal models [22]. The expression levels of other growth factors which are crucial for wound healing of tissue such as HIF-1α, VEGF receptor, stromal-derived factor-1 (SDF-1), and CXC chemokine receptor 4 (CXCR4) were also heightened and enhanced both cell survival and proliferation rates, while levels of pro-inflammatory cytokines were reduced following hypoxic preconditioning of MSCs at 0.5% oxygen. Moreover, these preconditioned mesenchymal stem cells have demonstrated superior efficacy in suppressing microglial activity in the brain and promoting locomotor recovery along with healing capacity compared to cells cultured under normoxic conditions [23,24].

MSCs preconditioned with oxygen levels ranging from 1% to 7% and transplanted into a murine hindlimb ischemia model enhance the expression of HIF-1α, GRP78, and Akt, thereby promoting tissue repair and increased healing potential. Furthermore, preconditioning with 2% oxygen enhances the stimulation of prion protein (PrPC), which in turn activates PrPC-dependent JAK2 and STAT3 signaling routes [24,25]. This activation enhances superoxide dismutase activity, inhibiting MSC apoptosis due to oxidative stress and facilitating functional recovery and improved healing mechanisms in ischemic tissue. Scientific literature indicates that preconditioned MSCs significantly upregulate glucose 6 phosphate transporter levels through HIF-1α and aryl hydrocarbon receptor (AhR) activation [26,27]. Several studies have highlighted the function of HIF regulatory proteins in regulating microRNA (miRNA) stimulation under low-oxygen environments. Hypoxia also accelerates the proliferation rate of bone marrow-derived mesenchymal stem cells, with miRNA 210 playing a crucial role in this process through interactions with the HIF pathway [24,28].

Pharmacological or chemical agent preconditioning strategies: to accelerate wound healing potential of MSC-based regenerative medicine:

Drugs are used to cure diseases having therapeutic effects that can provide functional and structural stability to various organs, increasing overall quality and survival for the patients. Many research studies have been focused to find out the mechanisms of drugs responsible for protection of MSCs from any damage thus refining their application in regenerative medicine [29,30]. Preconditioning of the MSCs with drugs is the process that helps to develop a protective window against any ischemic injury and improved cellular properties like proliferation and wound healing potential during the transplantation of stem cells and activation of its endogenous machinery for the progression of regeneration [30,31]. In this regard several drugs have been proposed to have protective effects on MSCs although the high concentrations have deteriorating effect on MSCs in terms of impairment of its functions. Therefore, it is crucial to find out the appropriate concentration of drugs both in vivo and in vitro exerting preconditioning effects on MSCs and its function [32,33].

Talking about drugs having variable effects according to their concentrations, zoledronic acid has been stated in literature to inhibit the growth and bone-forming specialization of bone marrow-derived MSCs when used in higher concentrations [34], while having opposite effects when used in lower concentration without having any influence on immunomodulatory properties of the cells. Another drug used for preconditioning is sevoflurane that stimulates various proteins like VEGF, HIF-1a, HIF-2a, and pAkt/Akt while stops the induction of apoptosis, reduction in membrane potential of mitochondria, consequently, preserves the endurance and differentiation potential of MSCs after H/SD and enhance the healing property of tissue [35,36]. Regarding streptozotocin that is commonly used to develop animal models of diabetes, is believed to have severely worsening effects on MSCs isolated from the models, these cell lack proliferating and angiogenic functions. To reverse these effects, OT has been used and showed significant regenerative capacity and specialization potential of these MSCs in living organisms. Similarly, induction of diabetes derived MSCs conditioned by OT demonstrated improved cardiac function with decrease fibrosis and increase heart tissue healing in myocardial infarction model of rats [37,38]. Having complicated procedures to enhance cardiac and neural differentiation, pharmaceuticals have been utilized to boost the differentiation process of MScs. In the model of cerebral ischemia, pretreatment with melatonin enhances the survival of transplanted MSCs and safeguards brain function from damage and healing mechanism by stimulation of the ERK signaling route [39,40].

The role of small molecules has been considerable in regenerative medicine in the development of iPSCs from somatic cells, contributing well in both cell and tissue engineering. Various studies probe the role of small molecules, such as BAY 11-708 has been studied and reported to block the pro-angiogenesis and antiapoptotic function of MSCs by inhibition of NF-jB [40]. Another molecule, LL37 that has endogenous antimicrobial effect, displayed substantial enhancement in the proliferation and migration of MSC by activating MAPK signaling pathway and enhance wound healing potential of tissues [41,42]. Alternatively, JI-34, a growth hormone-releasing hormone agonist, has been demonstrated to improve the differentiation and engraftment of MSCs in vitro on preconditioning. Since widespread use of small molecules has been in practice as preconditioning agents, they still constitute a very small percentage of all the active factors used. There is an intense need to develop many small molecules for the protection and improved cellular function and healing potential of MSCs [44,45,46].

Environmental factors and materials, preconditioning: for modulating MSC fate in wound healing potential:

For in vitro experiments, certain physical factors are detrimental to MSC functions and healing mechanisms. However, numerous studies suggest that these factors can actually enhance proliferation and differentiation both in vitro and in vivo. For instance, research has demonstrated that extremely low-level lasers are particularly effective in refining and accelerating proliferation and healing rates [47]. They achieve this by increasing the proportion of cells in the S-phase and promoting mitochondrial biogenesis through enhanced nitric oxide production in MSCs. Additionally, these lasers enhance the migration capability of MSCs by activating the ERK1/2 and FAK signaling routes, while also upregulating the stimulation of growth factors like HGF and PDGF which promotes cell proliferation and increase wound healing potential. Another promising approach involves GFc7, a nano-chelating nanocomplex, which significantly boosts cell proliferation and the expression of genes associated with pluripotency and proliferation. It also enhances homing markers and strengthens antioxidative defenses [49,50]. Meanwhile, selenite has been identified as beneficial for improving osteoblastic proliferation and differentiation in MSCs and enhance healing property. It achieves this by inhibiting the ERK signaling pathway stimulation, reducing glutathione for ROS generation, and enhancing the role of glutathione peroxidase, thereby providing antioxidant protection. To foster greater biochemical and biomechanical signals, researchers advocate for creating a three-dimensional microenvironment that mimics the physical conditions conducive to MSC proliferation and culture. Such environments facilitate robust cell-cell interactions and provide ample space for MSC propagation and improve the healing properties of MSCs [51].

Conclusion

Although cultured stem cells are extensively utilized in various cellular transplantation processes, their beneficial properties are often compromised due to low survival rates and high apoptosis. Several strategies such as preconditioning, genetic alterations, and diverse coculture techniques have been validated and thoroughly studied. The biological properties and functions of MSCs can be enhanced through different preconditioning methods both in vitro and in vivo, significantly improving their restorative and reparative efficiency in injured and diseased models. Despite the use of MSC transplantation in numerous clinical trials, further research is needed to fully understand how these preconditioning methods can influence and enhance the various biological activities and healing properties of transplanted MSCs. This knowledge will help make MSC-based therapies more effective and widely applicable in regenerative medicine and for treating various severe diseases in the future.

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Authors' contributions

NR drafted the review, authored the article, created the graphs, and supervised the manuscript's development. NZ contributed to the writing and edited the review. MZ and AM offered insights on the review's scope and content and assisted in the literature search. All authors reviewed and approved the final manuscript.

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Disclosure of interests

The authors declare that they have no financial or personal conflicts of interest.

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