

89874_Auto_Edited-check.docx

Name of Journal: *World Journal of Stem Cells*

Manuscript NO: 89874

Manuscript Type: EDITORIAL

Use of priming strategies to advance the clinical application of mesenchymal stromal/stem cell-based therapy

Miceli V. New strategies to improve MSC-based therapy

Abstract

Mesenchymal stromal/stem cells (MSCs) have garnered significant attention in the field of regenerative medicine due to their remarkable therapeutic potential. MSCs play a pivotal role in maintaining tissue homeostasis and possess diverse functions in tissue repair and recovery in various organs. These cells are characterized by easy accessibility, few ethical concerns, and adaptability to *in vitro* cultures, making them a valuable resource for cell therapy in several clinical conditions. Over the years, it has been shown that the true therapeutic power of MSCs lies not in cell engraftment and replacement but in their ability to produce critical paracrine factors, including cytokines, growth factors, and exosomes (EXOs), which modulate the tissue microenvironment and facilitate repair and regeneration processes. Consequently, MSC-derived products, such as conditioned media and EXOs, are now being extensively evaluated for their potential medical applications, offering advantages over the long-term use of whole MSCs. However, the efficacy of MSC-based treatments varies in clinical trials due to both intrinsic differences resulting from the choice of diverse cell sources and non-standardized production methods. To address these concerns and to enhance MSC therapeutic potential, researchers have explored many priming strategies, including exposure to inflammatory molecules, hypoxic conditions, and three-dimensional culture techniques. These approaches have optimized MSC secretion of functional factors, empowering them with enhanced immunomodulatory, angiogenic, and regenerative properties tailored to specific medical conditions. In fact, various priming strategies show promise in the treatment of numerous diseases, from immune-related disorders to acute injuries and cancer. Currently, in order to exploit the full therapeutic potential of MSC therapy, the most important challenge is to optimize the modulation of MSCs to obtain adapted cell therapy for specific clinical disorders. In other words, to unlock the complete potential of MSCs in regenerative medicine, it is crucial to identify the most suitable tissue source and develop *in vitro* manipulation protocols specific to the type of disease being treated.

Key Words: Mesenchymal stromal/stem cells; Therapeutic properties; Paracrine effects; Cell priming; Cell-free therapies; Regenerative medicine

Miceli V. Use of priming strategies to advance the clinical application of mesenchymal stromal/stem cell-based therapy. *World J Stem Cells* 2023; In press

Core Tip: Mesenchymal stromal/stem cells (MSCs) offer important therapeutic effects in the field of regenerative medicine. Their key role lies in the production of paracrine factors that modulate tissue environments and allow their repair following insults. Recently, MSC-derived products such as exosomes and conditioned media are replacing whole MSCs in clinical applications. In this regard, to optimize the results of MSC-based treatment, researchers have explored priming strategies in order to enhance MSC properties. Realizing the full potential of MSC therapy depends on identifying the right tissue source and developing priming strategies specific to the disease being treated.

INTRODUCTION

Over the years, mesenchymal stromal/stem cells (MSCs) have emerged as an important therapeutic tool in the field of regenerative medicine^[1-4]. These versatile multipotent adult stromal/stem cells play a crucial role in maintaining tissue homeostasis under both physiological and pathological conditions. In fact, MSCs possess the remarkable ability to influence their surroundings by differentiating, attracting supporting cells, and orchestrating central processes for tissue regeneration^[5,6]. Together, the multifaceted potential of MSCs shed light on their role as key regulatory elements in the complex mechanisms governing tissue repair/recovery in several tissues, including the intestine^[7], skin^[8], and skeletal muscle^[6], where MSCs exhibit diverse functions, either supporting high cellular turnover or facilitating regeneration following injury.

These discoveries offer a strong motivation for investigating the potential of MSCs as a cellular therapeutic product to enhance tissue injury responses in various diseases^[9-14].

MSCs show high accessibility, minimal ethics-related concerns, and great adaptability to *in vitro* cultures for expansion^[15]. Moreover, these cells possess immune privilege attributed to their low expression of CD40, CD80, CD86, and major histocompatibility complex I (MHC I), along with the absence of MHC II expression^[16,17]. These attributes make these cells a highly valuable resource for developing new cell therapies in the field of regenerative medicine.

MSCs are present in various tissues, including bone marrow^[18], adipose tissue^[19], umbilical cord^[14], dental pulp^[20], and placenta^[21]. In these diverse tissue environments, MSCs interact with different cell types, such as epithelial cells, endothelial cells, immune cells, and stromal cells, showing immunomodulatory, angiogenic, pro-trophic, and anti-oxidative properties^[22-25]. Their adaptability and therapeutic potential make them promising candidates for addressing a wide range of clinical disorders, including cardiovascular, neurodegenerative, immune, lung, liver, kidney, and orthopedic diseases. Notably, it has become increasingly evident that the true therapeutic power of MSC therapies lies not in engraftment and cell replacement but rather in their ability to produce critical paracrine factors that modulate the tissue microenvironment and facilitate repair and regeneration processes. Indeed, these cells are able to produce crucial functional factors, such as cytokines, growth factors, and exosomes (EXOs), which can mediate their therapeutic effects^[26-28]. Hence, given the regenerative potential and trophic properties inherent in certain MSC-derived products, such as the conditioned medium and/or EXOs, these products have arisen as potential therapeutic tools with a wide range of applications. Consequently, they are undergoing extensive evaluation for potential medical use^[9,12,29-32]. The clinical utilization of MSC-derived products must be considered for their advantages, particularly in contrast to concerns related to the prolonged use of MSCs and the associated risks of infectious disease transmission, such as viruses present in transplanted allogeneic cells^[33].

However, the therapeutic landscape of MSCs is not without its challenges and controversies. The efficacy of MSC-based treatments has yielded variable results in clinical trials, reflecting the complexity of intrinsic differences between cell-based

products and a lack of standardized methods for MSC production that affects their potency^[34-39]. The effects of MSCs vary based on the tissue source and the methods employed in their production and administration^[35,40,41]. Several studies have demonstrated that the composition of the MSC secretome can be modulated through the preconditioning of MSCs with cytokine treatments and hypoxia. Additionally, cultivating MSCs under specific culture systems, such as three-dimensional (3D) conditions, also influences their secretome. In response to MSC “priming”, the production of factors is switched towards a greater functional phenotype that results in an increase in MSC therapeutic effects^[3,27,42].

The field of research on MSCs is still very complex and is constantly evolving, emphasizing that the road to consolidating the use of MSCs as an effective cell therapy for various pathologies is still quite long. In this regard, promising approaches are being studied, among which MSC priming certainly represents one of the most hopeful strategies.

PRIMING STRATEGIES TO POTENTIATE THE THERAPEUTIC EFFECTS OF MSCS

In the last decade, the concept of priming or preconditioning MSCs has gained credibility as a means to enhance MSC therapeutic potential by modulating the secretion of paracrine factors and tailoring their actions to specific medical conditions^[3,27]. Similar to immune cells^[43], MSCs have been shown to memorize a stimulus after transitioning to a new environment^[44]. In this regard, MSCs can be primed to generate a short-term-memory effect and, mimicking microenvironmental stimuli, this strategy may be used *in vitro* to avoid the need for *in vivo* activation of the MSCs when aiming towards specific therapeutic activities. This approach has been widely explored in the context of immunomodulation^[45,46], tissue regeneration^[47,48], and even cancer interactions^[49], with each priming strategy offering a unique set of advantages and applications.

One of the principal priming strategies involves exposing MSCs to inflammatory molecules. Numerous studies reveal that the immunosuppressive properties of MSCs are not intrinsic but require priming by inflammatory factors. In fact, depending on the specific inflammatory conditions, the MSC phenotype can be polarized into MSC type 1, characterized by pro-inflammatory properties, or MSC type 2, with immunosuppressive capabilities^[50]. Various strategies have been implemented to modulate and enhance the secretion of immunomodulatory molecules in MSCs. The treatment of MSCs with inflammatory cytokines, including interferon- γ , interleukin (IL)-1 α / β , IL-6, tumor necrosis factor (TNF)- α , and IL-17, is shown to significantly enhance their immunomodulatory properties. This priming approach increases the production and secretion of key functional factors such as hepatocyte growth factor (HGF), transforming growth factor (TGF)- β , IL-6, prostaglandin E2 (PGE2), leukemia inhibitory factor (LIF), granulocyte colony-stimulating factor, IL-10, macrophage inflammatory protein (MIP)-1 α , indoleamine 2,3-dioxygenase (IDO), intercellular adhesion molecule, programmed death ligand (PDL)1-2, monocyte chemoattractant protein (MCP)-1, monokine induced by interferon-gamma, interferon-gamma-inducible protein 10, and MIP-1 β . These factors, in turn, empower MSCs with enhanced paracrine immunomodulatory properties, making them potent inhibitors of T cell proliferation and activators of anti-inflammatory M2 macrophage polarization^[27]. Moreover, treatment with inflammatory cytokines is shown to improve the immunomodulatory capabilities of extracellular vesicles (EVs) derived from MSCs, further highlighting the versatility of this priming strategy in the context of immunoregulation^[45,51].

Priming with hypoxia represents another pivotal approach to enhancing MSC functionality. Hypoxic preconditioning of MSCs is shown to stimulate the secretion of essential growth factors, such as vascular endothelial growth factor (VEGF) and HGF, which are crucial for angiogenesis and tissue regeneration^[52]. Under hypoxic conditions, MSCs activate signaling pathways, including the HIF-1 α -GRP78-Akt axis, leading to the overproduction of pro-angiogenic factors^[53]. This approach yields significant benefits in various acute injuries, including ischemia-reperfusion injury (IRI),

renal injury, and myocardial infarction^[3]. Moreover, hypoxic preconditioning is effective in promoting hepatic tissue regeneration, with increased expression of factors such as HGF and VEGF^[48,54]. This is particularly advantageous in cases of liver injury and fibrosis. Hypoxic MSCs also exhibit the ability to secrete functional EVs capable of stimulating tissue remodeling, contributing to tissue repair in cerebral tissue^[55]. In addition, hypoxic MSC-derived EVs show enhanced activity both *in vitro* and *in vivo*, especially in promoting angiogenesis on human brain microvascular endothelial cells. Interestingly, this effect appears to be mediated by microRNA (miRNA)-612^[56]. Therefore, several functional factors produced by hypoxia-primed MSCs are found to play a crucial role in stimulating angiogenic and regenerative activities, making this priming strategy a valuable tool to enhance MSC therapeutic effects for tissue recovery after acute injury.

Priming through 3D culture techniques offers an alternative approach to enhancing MSC therapeutic properties. This strategy involves the generation of MSC spheroids, which closely mimic the *in vivo* MSC niche and boost the functional phenotypic profile of MSCs. These spheroids exhibit superior trophic and immunomodulatory functionalities, driven by the paracrine secretion of functional factors with anti-inflammatory, angiogenic, anti-fibrotic, anti-apoptotic, and mitogenic properties^[30,51,57-59]. Comparative studies show that 3D culture of MSCs can modify their transcriptome profile, leading to the overexpression of genes that regulate proliferation, differentiation, immunomodulation, and angiogenic processes^[60]. These spheroids are found to secrete a plethora of regenerative and immunomodulatory factors, including stromal cell-derived factor-1 α , growth-regulated oncogene α , MCP-1/3, IL-4, IL-10, EGF, LIF, placental growth factor-1, VEGF-A/D, HGF, insulin-like growth factor 1, TNFAIP6, stanniocalcin 1, PDGFB, TGF- β , PGE2, and IDO. Such factors are involved in promoting tissue repair and regeneration, making 3D-cultured MSCs valuable for various applications in regenerative medicine^[27].

PRIMING STRATEGIES TO IMPROVE THE CLINICAL APPLICATION OF MSCS

The application of these priming strategies is not limited to basic research. They have found practical utility in the treatment of various clinical conditions (Table 1). For instance, in the context of chronic immune-related disorders, MSCs primed with pro-inflammatory cytokines demonstrate enhanced immunomodulatory properties, making them more effective in diseases such as colitis, autoimmune encephalomyelitis, and graft-versus-host disease (GVHD)^[61,66,102]. Notably, the priming of MSCs with IL-1 β shows promise in alleviating the side effects of sepsis, primarily by inducing macrophage polarization toward an anti-inflammatory M2 phenotype^[103]. Similarly, the use of TNF- α -primed MSCs attenuates symptoms of GVHD and peritonitis, with a demonstrated reduction in pro-inflammatory cytokines and an increase in anti-inflammatory factors^[67]. Moreover, the efficacy of MSCs primed with 3D culture conditions is evident in the treatment of diseases characterized by unresolved inflammation, as these spheroids overexpress TSG-6 and exhibit a more significant impact in reducing inflammation^[92].

The therapeutic potential of MSCs also extends to the treatment of acute injuries, where priming strategies can play a crucial role in boosting their regenerative capabilities. For instance, in cases of acute myocardial injury, hypoxic preconditioning significantly improves blood flow recovery, influences heart remodeling, and enhances the regeneration of ischemic tissues^[87,88]. These effects are attributed to the increased production of pro-survival and pro-angiogenic factors by hypoxia-primed MSCs, including HIF-1 α , ANGPT1, VEGF, Flk-1, Bcl-2, and Bcl-xL^[87]. Hypoxic MSCs demonstrate enhanced integration into damaged tissues, with improved survival, proliferation, and regenerative effects^[74]. In parallel, 3D-cultured MSCs show potential in both bone and cartilage repair, highlighting their capacity to stimulate tissue regeneration across various contexts^[98,99].

In recent years, the interaction between MSCs and cancer has also garnered considerable attention. Indeed, MSCs represent a crucial actor in the tumor microenvironment due to their ability to modulate the function/survival of both immune cells and tumor cells, with the final effects of promoting or inhibiting

cancer^[104]. Numerous studies have investigated the molecular mechanisms involved in the MSC-based modulation of tumor immunity, revealing that MSCs might either support or suppress tumor progression since many MSC factors can be produced differently in the tumor microenvironment^[104-106]. For instance, the cross-talk between MSCs and M1/M2 macrophages plays a pivotal role in regulating tumor progression^[107]. MSCs are shown to promote the shift from anti-tumorigenic M1 macrophages to pro-tumorigenic M2 macrophages, contributing to immune evasion and tumor growth^[108]. Moreover, the capacity of MSCs to express immune checkpoint molecules, including PDL1, further intensifies their role in immunosuppression, facilitating the evasion of host immune responses by cancer cells^[109]. On the other hand, various studies indicate that utilizing MSC-derived EVs housing anti-tumorigenic miRNAs might offer a novel therapeutic opportunity for MSC-based tumor therapy^[110].

In summary, priming strategies represent a versatile approach to managing the therapeutic potential of MSCs, tailoring their secreted factors and interactions to diverse clinical conditions. These strategies show great promise in regenerative medicine, immune-related disorders, and the complex interplay between MSCs and cancer (Figure 1). Through exposure to inflammatory molecules, hypoxic environments, 3D culture conditions, or other new priming strategies, MSCs can be transformed into highly specialized therapeutic tools, extending the possibilities for their application in various clinical settings and expanding our understanding of the dynamic role of MSCs in health and disease. The ongoing research in this field promises further advancements in the optimization of MSC-based therapies, offering new hope for patients suffering from a wide range of pathologies.

DISCUSSION

While research on MSCs is booming, as are their clinical applications, it is becoming increasingly important to understand the multiple properties of MSCs and how these can be optimally modulated to achieve the desired therapeutic effects. The use of MSC therapy, unfortunately, suffers from intrinsic biological variability, both due to the

source and inter-subject variability. On the other hand, these therapies might prove to be decisive in the treatment of certain so-called multifactorial pathologies where multiple molecular targets are involved, as in the case of inflammatory-related diseases^[111], including Alzheimer's and Parkinson's diseases^[112,113], cancer^[114], IRI^[13,115], and others. Due to the ability of MSCs to produce multiple functional factors capable of acting simultaneously on multiple targets, cell therapies based on the use of MSCs might be successful in the treatment of some such acute and chronic diseases for which effective treatments are currently lacking (Figure 2).

However, to achieve this goal, it will be necessary to understand how to modulate MSCs according to the specific dysfunction to be treated. In fact, while MSC immune inhibitory and pro-angiogenic effects may be suitable for various diseases in the field of regenerative medicine, the same properties might be disadvantageous in the treatment of some tumors. In the case of immune-mediated diseases such as GVHD or liver cirrhosis, MSCs with pronounced immunomodulatory capabilities might show enhanced therapeutic efficacy. Also, in the context of wound healing, MSCs displaying a well-balanced array of therapeutic attributes, encompassing immunomodulation, trophic stimulation, and angiogenic promotion, may be more efficacious.

CONCLUSION

It is true that MSCs from various sources possess unique therapeutic properties, but it is unthinkable that they can be extracted from any tissue and used as they are for various types of diseases. The only way to build an effective cell therapy based on MSCs is to first establish the most suitable source in terms of therapeutic efficacy with the least invasive strategy required for their isolation. Subsequently, appropriate *in vitro* manipulation strategies should be studied to promote their expansion and trigger specific therapeutic functions in order to establish MSC manipulation protocols specific to the type of disease to be treated. Our future goal should be to unlock the full potential of MSCs, fostering a deeper appreciation of their remarkable therapeutic capabilities and actively contributing to the ongoing progress of regenerative medicine.

10%

SIMILARITY INDEX

PRIMARY SOURCES

- | | | |
|----------|---|-----------------------|
| 1 | encyclopedia.pub <small>Internet</small> | 122 words — 4% |
| <hr/> | | |
| 2 | www.ncbi.nlm.nih.gov <small>Internet</small> | 112 words — 4% |
| <hr/> | | |
| 3 | www.frontiersin.org <small>Internet</small> | 20 words — 1% |
| <hr/> | | |
| 4 | Vitale Miceli, Giovanni Zito, Matteo Bulati, Alessia Gallo, Rosalia Busà, Gioacchin Iannolo, Pier Giulio Conaldi. "Different priming strategies improve distinct therapeutic capabilities of mesenchymal stromal/stem cells: Potential implications for their clinical use", World Journal of Stem Cells, 2023 <small>Crossref</small> | 18 words — 1% |
| <hr/> | | |
| 5 | James Q. Yin, Jun Zhu, James A. Ankrum. "Manufacturing of primed mesenchymal stromal cells for therapy", Nature Biomedical Engineering, 2019 <small>Crossref</small> | 15 words — 1% |

EXCLUDE QUOTES ☐ ON

EXCLUDE BIBLIOGRAPHY ☐ ON

EXCLUDE SOURCES

EXCLUDE MATCHES

< 15 WORDS

< 15 WORDS